Vol. XXI September, 1944 No. 5

CEREAL CHEMISTRY

Published bi-monthly by the American Association of Cereal Chemists
at Prince and Lemon Sts., Lancaster, Pa.

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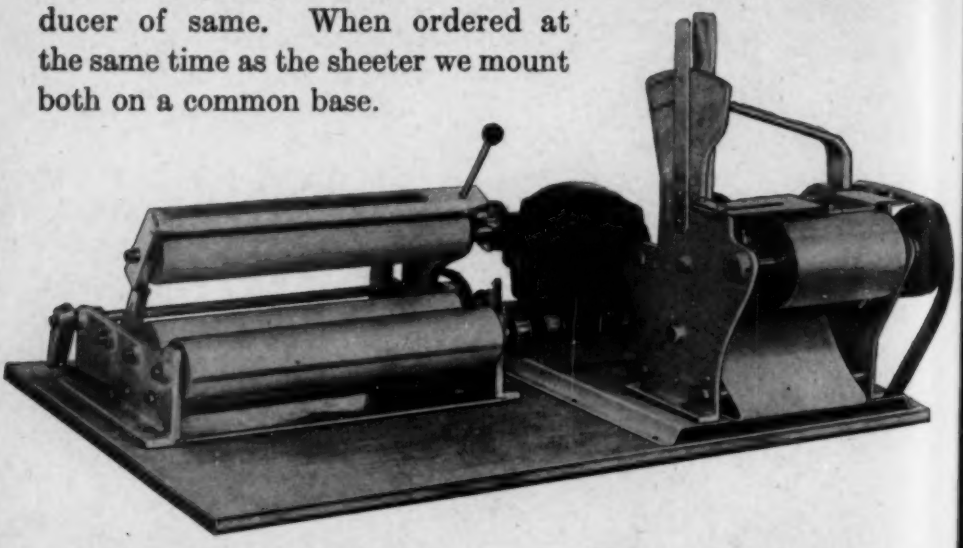

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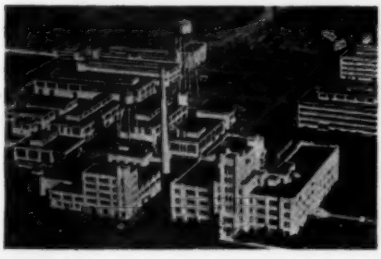

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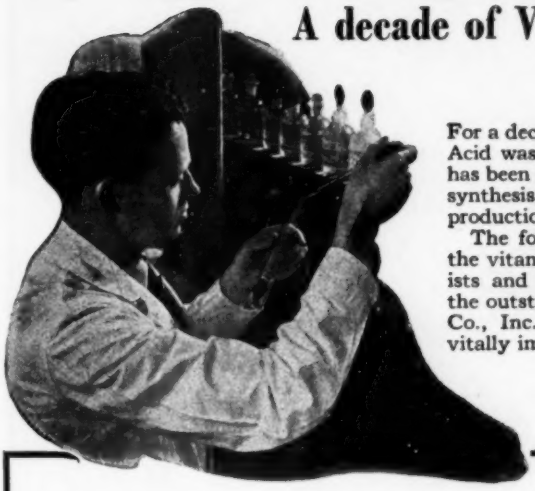

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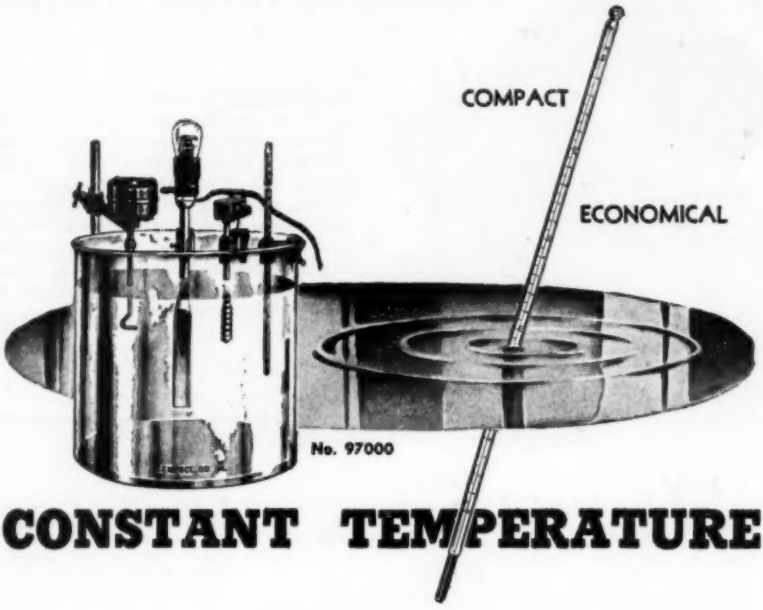

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CEREAL CHEMISTRY

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GELATINIZATION STUDIES UPON WHEAT AND OTHER STARCHES WITH THE AMYLOGRAPH [1]

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(Presented at the Annual Meeting, May 1942; received for publication January 19, 1944)

In recent years, considerable attention has been given to the development of convenient tests for the detection of excess amylase activity in relation to its undesirable effects on the crumb characteristics of baked goods. Kozmin (1933) and Molin (1932, 1934) were apparently the first to point out that excessive enzymic degradation of starch during baking is responsible for the production of bread with a moist sticky crumb of poor eating quality because of the decreased ability to bind water set free by coagulation of the gluten proteins.

The control of alpha-amylase activity appears to be of particular importance in the manufacture of breads made largely from rye flours, and Brabender (1937) described a recording viscosimeter for evaluating the baking quality of these flours. This apparatus, known as the amylograph, provides a continuous automatic record of the changes in viscosity of a flour-water suspension as the temperature is increased at a uniform rate. The increase in viscosity which takes place upon gelatinization of the starch is opposed by the liquefying action of the amylase present and the height of the curve at maximum viscosity is considered an index of amylase activity (Brabender, 1937; Brabender, Mueller, and Köster, 1937). The amylograph has been applied to flour quality studies by Schmidt and Scholz (1938); Brabender, Mueller, and Heide (1938); and Scholz (1940). Other torsion-type instruments have been developed which, like the amylograph, permit a study of the relative viscosity changes which occur in starch suspensions with increases in temperature (Caesar, 1932; Caesar and Moore, 1935; Radley, 1940; Barham, Wagoner, and Reed, 1942). The course of starch gelatinization may also be followed by measuring the increase in light transmission of starch suspensions upon heating (Cook and Axtmayer, 1937).

[1] Paper No. 2178, Scientific Journal Series, Minnesota Agricultural Experiment Station. Condensed from a thesis presented by Charles A. Anker to the faculty of the graduate school in partial fulfillment of the requirements for the degree of Master of Science, December, 1942.

The recent studies of Hollenbeck and Blish (1941) justify the use of starch liquefaction as a measure of the dextrinization of starch by alpha-amylase. In testing wheat or rye flours, however, several variables, such as starch content, inherent starch characteristics, extent of mechanical injury of the starch, and pH, may materially influence the paste viscosity in addition to differences in the extent of liquefaction which results from variations in alpha-amylase activity. Although there may be definite limitations in the general use of the amylograph as an index of the relative alpha-amylase activity of flours, it would appear to provide a convenient means for investigating differences in the pasting properties of starches from various sources; also for studying the relative resistance of different starches to alpha-amylase and the effects of different processing treatments used in the starch industry.

The studies reported in this paper represent preliminary investigations designed to determine the possible utility of the amylograph in flour and starch technology. In addition to experiments related to the technique of operation, the amylograph was employed in studies of the relative gelatinization characteristics of wheat, corn, and potato starch at various concentrations, of the effect of storage on the paste viscosity of wheat starch, and of various agents on pasting properties.

The Amylograph and Its Operation

Description and Operation. The amylograph is a torsion viscosimeter which automatically records the resistance to shear offered by a flour or starch suspension as the temperature of the suspension is increased at a constant rate of approximately 1.5°C per min. The cylindrical, tinned-brass bowl (operating capacity 500 ml) in which the suspension is placed contains eight fixed, vertical pins and is rotated at the rate of 75 rpm in an electrically heated air bath by means of a synchronous motor which also operates the kymograph and the device for controlling the rate of temperature increase. The customary viscosimeter bob is replaced by seven metal pins attached to a circular metal disc around a central shaft, which is connected at its upper end to a coiled-wire spring; this, in turn, is fastened to the lever and pen of the kymograph. The rotation of the bowl forcing the suspension past the pins exerts a stirring effect and the frictional resistance causes the free-moving pins to rotate on their central axis against the resistance of the coil-spring. The extent of rotation is recorded by the kymograph in arbitrary units ranging from zero to 1,000 with rulings at 20-unit intervals; time rulings are provided for 1-min intervals.

The heater circuit is controlled by a contact mercury thermometer which functions as an ingenious thermoregulator. Provision is made for elevating the upper contact wire of the thermometer by means of the

synchronous motor, at a rate which requires a temperature rise of about 1.5°C per min to complete the circuit between the two contacts. The movement of the contact may be arrested at any desired temperature by means of a clutch which disengages the driving mechanism. A crank is provided for the manual setting of the contact at any desired temperature. The head of the amylograph, which supports the thermoregulator and shaft carrying the movable pins, may be raised by a lever to permit these parts to be swung out of position for convenient filling and removal of the bowl.

The operation of the amylograph has been described by Brabender, Mueller, and Köster (1937) and is quite simple. With starches, a convenient weight (usually 40–50 g) is placed in a 500 ml Erlenmeyer flask and 250 ml of distilled water at 25°C added; the flask is then stoppered and shaken to form a smooth suspension which is poured into the amylograph bowl. Wheat flours are mixed with the water in a small bowl by means of an egg beater in order to avoid lumping. An additional 200 ml of distilled water at 25°C is employed to rinse out the Erlenmeyer flask or bowl, and the rinsings are added to the suspension. The head of the amylograph is swung into position and the thermometer and movable pins are lowered into place. The upper contact of the thermometer is set at 25°C, the kymograph adjusted, and the control switch turned on. The temperature of the suspension is normally allowed to increase to 95°C when it is held constant. The apparatus is usually operated for a total elapsed time of 60 min.

Relation of Response to Shearing Stress. It was necessary, first, to ascertain whether the kymograph readings bore a linear relation to the force acting against the coil-spring of the instrument. An approximate test of the linearity of response was made in the following manner. The amylograph was completely assembled and the bowl left empty. A short lever was attached to the cover of the bowl and to this a strong cord was tied which passed over a small pulley and had an aluminum weighing pan fastened to the free end. The pulley was so arranged that the force applied to the cord was exerted at right angles to the lever at an amylograph reading of 500 units, that is, at the midpoint of the total angle (approximately 60°) described by the lever in covering the entire range of the instrument. With the writing arm adjusted to zero, weights ranging from 20 to 230 g in 10-g increments were placed on the pan and the amylograph units recorded. The data, given in Table I, deviate only slightly from a linear relationship. Some deviation would be expected since the applied force was not uniformly exerted at right angles to the lever. Also, the applied load tended to pull the shaft to one side, thereby increasing the friction against the bearings as the load became greater. It may be concluded that the

amylograph readings are essentially directly proportional to the resistance to shear of the medium under test.

Form and Evaluation of the Amylograph Curve. For convenience, the resistance to shear, as measured in arbitrary units by the amylograph, will be called "viscosity" throughout this paper. It is recog-

TABLE I

RELATION BETWEEN AMYLOGRAPH READING AND APPLIED LOAD[1]

Load	Amylograph reading	Load	Amylograph reading	Load	Amylograph reading
g	*B. U.*	*g*	*B. U.*	*g*	*B. U.*
20	90	90	465	160	775
30	155	100	510	170	810
40	215	110	560	180	850
50	250	120	600	190	890
60	315	130	650	200	930
70	365	140	690	210	960
80	410	150	730	220	990

[1] Recorded values apply to weights added and do not include the weight of the cord and pan.

nized that the resistance to shear or apparent viscosity depends upon several factors, such as the extent of aggregation of the granules, the extent of swelling (which not only influences viscosity by altering the volume relation between disperse phase and dispersion medium, but also by its influence on the degree to which the granules may be deformed under pressure), and the extent of granule disintegration or rupture, which not only changes the volume relation between disperse phase and dispersion medium but also the composition of the latter. Above certain limiting or critical concentrations which depend on such factors as the kind of starch, the extent to which it has been modified by pretreatment, and the pasting conditions, the viscosity of starch pastes is dependent upon the rate of shear, that is, such pastes exhibit anomalous or structural viscosity. These limiting concentrations are rather low for unmodified starch pastes: corn starch suspensions, for example, show anomalous viscosity at concentrations above about 2% when pasted at 90°C (Brimhall and Hixon, 1942). It is well known that starch pastes exhibit thixotropy, that is, they have gel properties when quiescent, become more fluid on the application of a shearing force, and again behave as gels when allowed to return to the quiescent state. Brimhall and Hixon (1942) state that the amylograph and consistometer "give results in which structural viscosity is subordinated to the resistance of the granules to crushing and the thixotropic characteristics of the pastes."

Several workers have established that when an aqueous starch suspension is gradually heated, the granules lose their characteristic birefringence before appreciable swelling occurs. Upon further heat-

ing, swelling becomes pronounced with a resulting increase in viscosity; in the absence of mechanical action, relatively little granule disintegration and solubilization of the starch takes place. Gallay (1936) and Gallay and Bell (1936) have concluded that the viscosity of a starch paste that has not undergone any severe pretreatment depends on the volume relation between disperse phase and dispersion medium and on

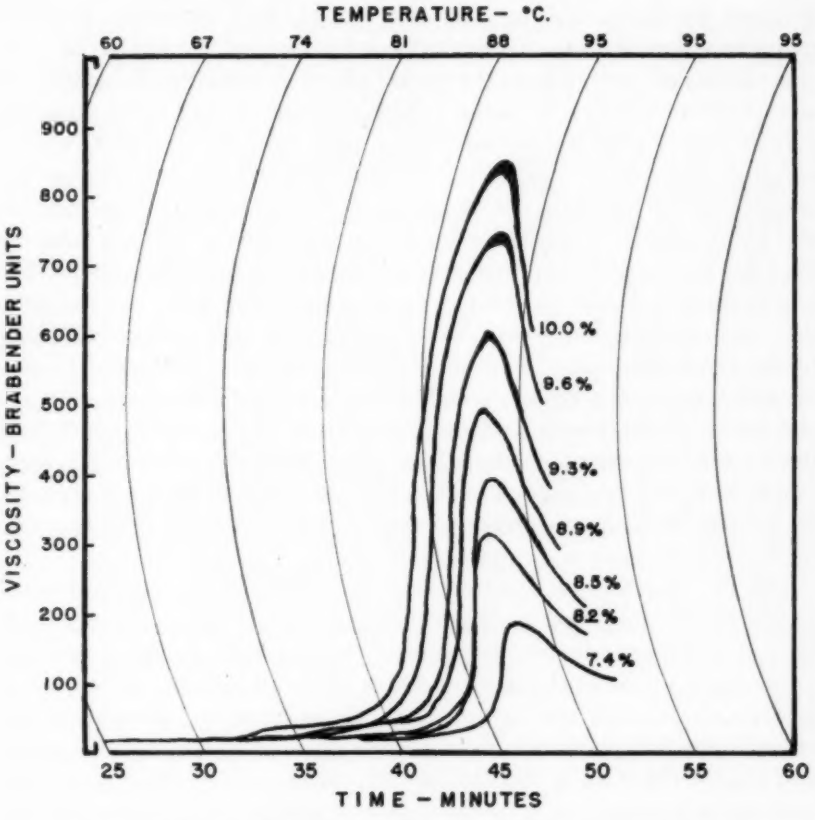

Fig. 1. Effect of wheat starch concentration on amylograph curve characteristics.

the deformability of the swollen granules. From studies of the viscosity changes which occur in corn and potato starch pastes on heating and stirring, Katz (1938) concluded that the heat gelatinization curve is a result of two opposing factors: one, the progressive swelling and hydration of the starch granule (which increases viscosity), and the other, the breakdown of the vesicle walls (which decreases viscosity). Schoch (1941) has pointed out that the viscosity of a boiled starch paste must be due largely to the presence of swollen aggregates or

fragments of granule structure since the viscosity markedly decreases upon autoclaving or violent mechanical agitation.

Representative amylograph curves made with commercial wheat starch suspensions of varying concentration are shown in Figure 1. The particular amylograph used in this study provided a temperature increase of 1.39°C per min. The general form of the gelatinization curve is similar to that obtained by Caesar (1932) with the consistometer, and by Barham, Wagoner, and Reed (1942) with their rotating cylinder viscosimeter. As pointed out by Caesar, the initial flat portion of the curve represents the period where any swelling is insufficient to register an increase in viscosity with the instrument; as the temperature is raised swelling becomes more and more pronounced, with a resultant increase in viscosity. Granule disintegration is not an important factor affecting the viscosity until swelling has progressed to the point where the granules become rather closely packed. As the packing becomes closer, the internal shearing stress increases with a concomitant increase in the extent of granule rupture. At the peak viscosity, swelling has nearly reached a limiting value and the influence of the small remaining increase in swelling is counterbalanced by any viscosity-decreasing factors which are operative. From this point on, the latter predominate, and further stirring and heating result in a decrease in viscosity. Although granule disorganization is the commonly accepted explanation of the sharp recession of the pasting curve, an increase in the permeability of the swollen granules may play a part in this phenomenon.

As the starch concentration increases, there is an appreciable decrease in the temperature at which the viscosity shows a measurable change, a marked increase in maximum viscosity, and a slight decrease in the temperature of the paste at which the peak viscosity is registered; moreover, the peak viscosity is more abrupt, and the subsequent decrease in viscosity is more rapid. Caesar (1932) and Barham, Wagoner, and Reed (1942) have reported similar findings. As the starch concentration is increased, a given degree of swelling will have a greater effect on the viscosity of the suspensions; consequently, a lower temperature and less swelling will be necessary to bring about a viscosity increase sufficient to be recorded by the amylograph. It is noteworthy that the amylograph is relatively insensitive to any swelling which occurs at temperatures below about 71°C. As pointed out by Caesar (1932), the lower paste temperature and the increase in slope of the down-gradient portion of the curve, as the starch concentration is increased, may be explained as being the result of greater granule rupture due to closer packing of the swollen granules.

As it is impractical to reproduce large numbers of gelatinization

curves, it is necessary to adopt some simple means of presenting their most significant characteristics in tabular form. For this purpose, three measurements can be readily taken from the curves: (1) the temperature at which the first perceptible increase in viscosity occurs; this has been conveniently called the temperature of transition by Cook and Axtmayer (1937); (2) the maximum viscosity; and (3) the temperature at which maximum viscosity is attained.

Precision of Amylograph Values. As an index of the precision of the amylograph, an analysis was made of the data for 69 sets of duplicate values obtained in connection with the various studies reported in this paper. The results were as follows:

Variable	Mean value	Mean difference between duplicates	Standard error (single determination)
Temperature of transition, °C	74.4	0.64	0.95
Paste temperature at maximum viscosity, °C	88.5	0.20	0.30
Maximum viscosity, Brabender units	59.5	6.2	9.3

Because of the gradual initial increase in viscosity, the error in estimating the temperature of transition from the curve is much higher than that involved in estimating the paste temperature at maximum viscosity. Considering the magnitude of the values, the replicate error for maximum viscosity is very satisfactory, especially since the kymograph paper is only ruled to 20 units; on the basis of the mean value of this variable, the error is 1.6%.

Effect of Variations in Technique. Since the viscosities of the wheat starch suspensions shown in Figure 1 did not increase until temperatures of from 71° to 83°C (depending upon the concentration) were reached, after 33 min or more of heating, it appeared that the test might be speeded up by employing a starting temperature just below the temperature of transition. The influence of starting temperature on curve characteristics was studied by preparing, in duplicate, five suspensions (containing 9.1% of wheat starch) at temperatures varying between 25° and 65°C; gelatinization curves were made with the contact thermometer set initially at the respective temperatures employed in preparing each suspension. The mean results, summarized in Table II, show a marked increase in maximum viscosity with starting temperatures exceeding 45°C. These results emphasize the importance of maintaining a uniform rate of heating throughout the entire course of the swelling and gelatinization process if consistent results are to be obtained. In fact, uniformity of technique in preparing the suspensions is also important. Allowing the prepared suspensions to stand

TABLE II

EFFECT OF STARTING TEMPERATURE ON THE AMYLOGRAPH CURVES FOR 9.1% SUSPENSIONS OF COMMERCIAL WHEAT STARCH

Starting temperature	Temperature of transition	Paste temperature at max. viscosity	Maximum viscosity
°C	°C	°C	B. U.
25	68.8	90.5	658
35	67.0	91.0	690
45	72.9	90.8	692
55	65.6	90.8	778
65	73.3	91.4	816

for 15 min before commencing a test was found to increase slightly the temperature of transition and the maximum viscosity.

In subsequent experiments, the curves were started at 25°C immediately after the suspensions were prepared. Employing the standard technique of heating to 95°C and then holding the temperature constant at this value for a total elapsed time of 60 min, wheat starch suspensions (9.1% starch) lost 23 to 24 g of water by evaporation.

Gelatinization Characteristics of Corn, Wheat, and Potato Starch

To secure an index of the difference in amylograph curve characteristics for unmodified commercial corn, wheat, and potato starch, curves were made with these starches at suitable concentrations to give maximum paste viscosities which fell within 700 and 800 Brabender units. The results of mean determinations are given in Table III.

TABLE III

GELATINIZATION CHARACTERISTICS OF POTATO, CORN, AND WHEAT STARCH

Starch	Starch concentration	Temperature of transition	Paste temperature at max. viscosity	Maximum viscosity
	%	°C	°C	B. U.
Potato	5.0	64.6	88.9	720
Corn	7.0	73.7	90.0	745
Wheat	10.0	70.2	91.2	830

The potato starch gave a viscosity increase at a much lower temperature than the corn and wheat starch and the maximum paste viscosity was reached at a slightly lower temperature. The viscosity of the potato starch rose very rapidly after swelling began and exhibited a much broader maximum and lower rate of decrease than that of corn or wheat starch.

To secure a comparison of the relative paste viscosities of the three starches, amylograph curves were made with each starch at a series of concentrations. The results are given in Table IV. As shown in Figure 2, when the logarithms of the maximum viscosities are plotted

TABLE IV

EFFECT OF CONCENTRATION ON MAXIMUM VISCOSITY OF POTATO, CORN, AND WHEAT STARCH

Potato starch		Corn starch		Wheat starch	
Starch concentration	Maximum viscosity	Starch concentration	Maximum viscosity	Starch concentration	Maximum viscosity
%	*B. U.*	%	*B. U.*	%	*B. U.*
2.17	135	5.86	220	8.54	230
2.60	210	6.64	320	9.27	350
3.02	290	7.02	405	10.00	485
3.43	390	7.22	420	10.71	570
3.85	510	7.41	470	11.42	710
4.05	590	7.79	545	12.11	875
4.26	660	8.16	610		
4.66	825	8.54	720		
		8.91	810		

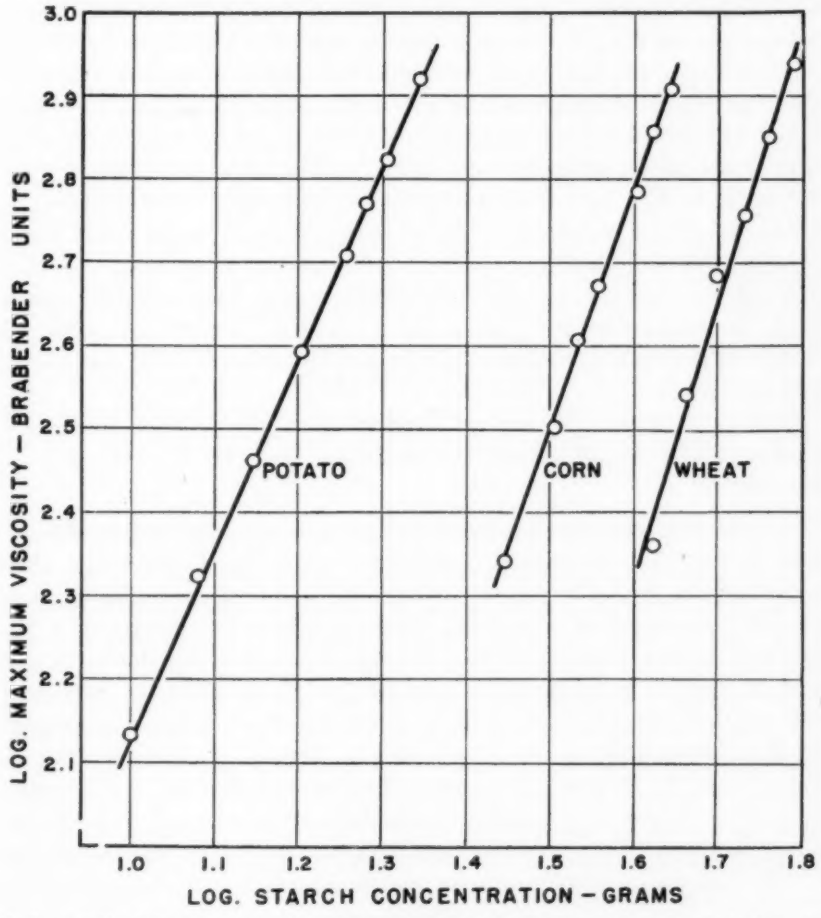

Fig. 2. Relation between concentration and maximum paste viscosity of potato, corn, and wheat starch.

against the logarithms of starch concentration a straight line results for each starch. These data show the wide differences in paste viscosity of the three starches at equivalent concentrations.

Mathematical Relation between Starch Concentration and Paste Viscosity

Brimhall and Hixon (1942) have recently reviewed the various equations proposed for expressing the relation between viscosity and the volume or the concentration of the starch granules. In studies with a series of wheat starches, Rask and Alsberg (1924) found that by plotting the logarithms of the viscosity of the pastes, as determined at 90°C with a Stormer viscosimeter, against starch concentration (2.8%–5.8%), a straight line resulted. Brimhall and Hixon, in determining the hot paste viscosities of unmodified corn starches, in a capillary viscosimeter, found that the logarithmic relation of Rask and Alsberg held only over certain ranges of concentration and pressure.

The linear relation between the logarithms of maximum viscosity and logarithms of starch concentration noted in the present work was found to hold with unmodified potato, corn, and wheat starch over all ranges in concentration investigated. This relation is represented by the formula $y = kx^n$. Should further investigation establish that it holds generally for various native and modified starches, it would be of considerable practical significance. By making a series of viscosity determinations over a range of concentrations, regression equations could be established for each starch type, from which the paste viscosity corresponding to any desired concentration could be computed, or vice versa.

An explanation [2] of the observed relationship between maximum viscosity and initial starch concentration may be reached if it is assumed:

(1) that during the gelatinization process, the ungelatinized granules A form highly swollen gelatinized granules B which are then eventually ruptured as a result of shearing action, thereby losing most of their incorporated water and forming relatively nonhydrated disintegrated granules C; (2) that the viscosity increment due to A and to C is small or insignificant as compared with the viscosity increment due to B; (3) that the viscosity-concentration relationship with respect to B is approximately described by the Arrhenius equation: that is $\log \eta_r = K[B]$ (where $[B]$ denotes the concentration of B); (4) that $\eta_s >> \eta_o =$ where $\eta_s =$ viscosity of solution and $\eta_o =$ viscosity of solvent. The relative viscosity η_r would then be approximately proportional to η_s

[2] The authors are indebted to D. R. Briggs, Division of Agricultural Biochemistry, University of Minnesota, for the explanation referred to.

and the value η_s could be substituted for η_r in the Arrhenius equation; that is, $\log \eta_s = K[B]$; (5) that the process A → B, involving the taking up of water by the granule, is a first-order process. At the end of a given time, the amount of B present would then be directly proportional to the initial amount of A present. This process could be expected to be of the first order as long as there was sufficient water present so that no competition occurred between granules for the water; (6) that the process B → C, involving the rupture or disintegration of the gelatinized granules, is a second- or higher-order process. This would be expected since the rate of disintegration should be a function of the shearing action on the swollen granules, and the intensity of this shearing action is a function of the viscosity, which in turn is a function of the concentration of B.

On the basis of these assumptions, the amount of B formed in a given time after the process began would be proportional to the initial concentration of A; however, as the initial concentration of A is increased, the maximum amount of B would be reached in a shorter time, and the value of the maximum attained for B would vary logarithmically with the initial concentration of A (or to the amount of B formed in each comparable unit of time after the process A → B is initiated):

thus, $$[B]_{max.} = K \log [A]_{initial}$$

and $$\log \eta_{smax.} = K'[B]_{max.}$$

then $$\log \eta_{smax.} = K'K \log [A]_{initial};$$

this is the observed relationship. The amylograph curves also show a slight displacement of the maxima toward lower time values as the initial starch concentration is increased (see Fig. 1). This is not marked because of the very short time interval which is required for the entire process: A → B → C.

Effect of Various Agents on the Pasting Properties of Starch

Effect of Flour Proteins upon Amylograph Curves for Wheat Starch. In interpreting peak viscosities obtained with suspensions of wheat flours as a measure of flour amylase activity, it must be assumed that the viscosities are not materially influenced by other variables. The marked influence of starch concentration on maximum viscosity indicates that this variable would have to be carefully controlled. In wheat flours, however, a decrease in starch is accompanied by an increase in protein; in flours of equivalent extraction the sum of these two constituents may be regarded as being approximately equal. It was therefore of interest to determine the effect of complementary variations in starch and protein content on maximum viscosity.

Gluten was washed from an undiastated hard red spring wheat flour, dried in thin layers at 25°C under vacuum, and finely ground; it contained 82% of protein (dry matter basis). Two series of amylograph curves were made. One series comprised mixtures of this gluten and commercial wheat starch in which the protein content was varied from 6 to 16% in 2% increments; the other series was made with starch alone, employing the respective quantities present in the various starch-gluten mixtures. In making the curves for the mixtures, the gluten was hydrated for 2 hr in a 100-ml portion of the water used to suspend the starch.

Since flour amylases are, in part, absorbed on, or occluded in, the gluten fraction, the amylase activity of the gluten-starch mixtures would be expected to increase with increasing gluten content. The diastatic activities of the starch, and of starch-gluten mixtures containing 6 and 16% protein, were determined as outlined in Cereal Laboratory Methods (4th ed., 1941); the respective mean values were 8, 16, and 18 units. It may be assumed that these relatively small differences in diastatic activity would not materially influence the gelatinization characteristics, especially since they may be ascribed chiefly to beta-amylase activity.

TABLE V

EFFECT OF STARCH CONCENTRATION AND ADDED GLUTEN ON MAXIMUM PASTE VISCOSITY

Protein added as gluten	Maximum paste viscosity [1]	
	Starch-gluten mixture	Starch
%	*B. U.*	*B. U.*
0	952	952
6	760	728
8	702	640
10	630	558
12	600	505
14	558	452
16	500	390

[1] The total dry weight of starch and protein was maintained at 46 g in 450 ml of distilled water. The values recorded in the starch column were obtained by gelatinizing starch suspensions which contained the same concentration of starch as was present in the starch-gluten mixtures.

The mean maximum viscosities recorded in Table V show, as anticipated, that the substitution of gluten proteins for an equivalent weight of starch decreases the maximum paste viscosity. At equal starch concentrations, however, the presence of gluten increases viscosity. These results imply that, in studying alpha-amylase activity of flours with the amylograph, the effect of variations in protein and starch content cannot be eliminated by weighing the samples on a constant-protein, constant-starch, or constant-protein-plus-starch basis.

Effect of pH on Amylograph Curves for Wheat Starch. The effect of pH on the amylograph curve for commercial wheat starch was investigated over the normal range for fermenting doughs (pH 5.2–6.7). Two buffer mixtures were employed, namely, 0.05*M* bimaleate buffers (prepared according to Temple, 1929), which covered the entire range desired, and 0.05*M* citrate buffers (prepared according to Kolthoff and Vleeschouwer, 1926), which covered the pH range of 5.2–6.0. The curves were made with 45 g (dry basis) of commercial wheat starch and 450 ml of the respective buffer solutions. A glass electrode was used in determining the pH of the suspensions. The mean results of duplicate determinations are summarized in Table VI.

TABLE VI

EFFECT OF pH AND BUFFER COMPOSITION ON THE AMYLOGRAPH CURVE CHARACTERISTICS OF COMMERCIAL WHEAT STARCH [1]

pH of suspension	Temperature of transition	Paste temperature at max. viscosity	Maximum viscosity
	°C	°C	*B. U.*
Control			
4.30	82.7	93.2	880
Bimaleate buffer mixture			
5.26	79.2	92.8	932
5.72	79.2	93.3	870
6.08	79.2	93.8	836
6.42	79.9	94.2	780
6.71	79.2	94.9	760
Citrate buffer mixture			
5.32	81.3	93.8	910
5.79	82.0	94.5	830
6.13	79.2	95.2	750

[1] Suspensions contained 9.1% starch.

The maximum paste viscosity for each buffer, respectively, decreased in linear fashion with an increase in pH; the decrease was greater for the citrate than for the bimaleate buffer. However, the control sample, which was the lowest in pH, gave a greater peak viscosity than several of the suspensions which were buffered at higher pH values. This, together with the differences in the effect of the two buffer solutions, leads to the suggestion that the results may be due to the effects of ion adsorption on the permeability of the granule.

Effect of Alpha-Amylase on the Gelatinization Curve for Various Wheat Starches. The effect of alpha-amylase activity on the form of the amylograph curve for commercial wheat starch was followed over a diastatic activity range of 17 to 600 maltose units, as determined by the regular A. A. C. C. procedure for wheat flour (Cereal Laboratory Methods, 4th ed., 1941). Takadiastase (undiluted; Parke Davis and Co.) was used as a convenient means of increasing the alpha-amylase

activity of the starch. The quantity of takadiastase varied between 0 and 2% of the weight of the starch and was dispersed in the water used in preparing the starch suspensions. To secure full advantage of the amylograph scale range, the curves were made with 60 g of starch (dry basis) and 450 ml of distilled water or takadiastase dis-

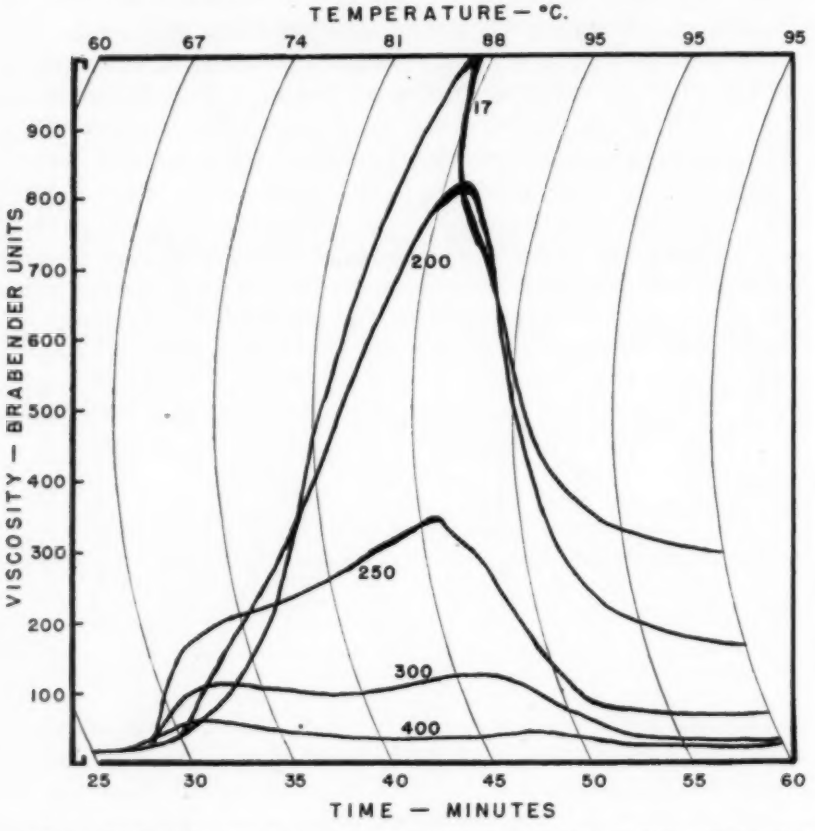

Fig. 3. Amylograph curves for commercial wheat starch brought to diastatic activities of 17, 200, 250, 300, and 400 maltose units (dry basis) by addition of takadiastase. The starch suspensions contained 11.8% starch.

persion. The pH of the various suspensions was 4.3. The maltose content of the gelatinized pastes was determined by the ferricyanide procedure employed in determining diastatic activity, immediately following the 60-min gelatinization period.

Figure 3 shows representative amylograph curves for commercial wheat starches brought to diastatic activity levels of 17 to 400 maltose units. Mean curve readings for the entire series, together with the quantities of maltose produced during gelatinization, are given in Table VII.

These data show the marked liquefying action of alpha-amylase. As measured by the diastatic activity of the starch-takadiastase mixtures, the effect of alpha-amylase on paste viscosity is curvilinear. As the maximum viscosity decreased, the peaks became less sharp than in the instance of lower peak viscosities due to a decrease in starch concentration (Fig. 1). The curves for wheat starch with diastatic activities of 300 and 400 units exhibited two ill-defined peak viscosities at widely different paste temperatures.

TABLE VII

EFFECT OF ADDED TAKADIASTASE ON AMYLOGRAPH CURVE CHARACTERISTICS FOR COMMERCIAL WHEAT STARCH, AND ON MALTOSE PRODUCTION DURING PASTING

Diastatic activity of starch	Amylograph curve characteristics			Maltose produced [1]
	Temperature of transition	Paste temp. at max. viscosity	Maximum viscosity	
maltose units—mg/10 g	°C	°C	*B. U.*	*mg/10 g*
17	64.6	86.8	1,000	108
100	64.6	87.8	988	133
200	64.6	88.8	798	633
250	63.9	88.2	410	1,053
300[2]	63.9	90.0	133	2,400
400[2]	62.8	68.1	62	4,933
500	63.9	67.4	36	8,600
600	64.6	67.4	31	9,600

[1] Maltose expressed as mg of maltose per 10 g of starch produced during the 60 min required for amylograph test.
[2] Two peak viscosities were observed; the paste temperatures and maximum viscosities given are for the highest peak viscosities.

Increasing alpha-amylase activity had no significant influence on the temperature of transition but the paste temperature at maximum viscosity showed a sudden decrease when the diastatic activity of the starch was increased from 300 to 400 maltose units.

The liquefying effect of alpha-amylase next was investigated with wheat starches prepared in the laboratory from five commercially milled flours: a durum fancy patent, southwestern winter wheat patent, hard red spring wheat patent, soft wheat patent, and a Minnesota winter wheat patent. Each flour was mixed to a stiff dough with water; after standing in water at 15°C for one hr, the gluten was washed out with tap water and the starch recovered from the wash water by centrifuging. After washing three times with distilled water, the starch was dried at room temperature in a current of air. Three levels of takadiastase (0.042, 0.210, and 0.525%), which gave diastatic activity values of 100, 200, and 300 maltose units with the commercial wheat starch previously studied, were added to each starch and the diastatic activities then determined. Amylograph curves were made

in duplicate employing 45 g of starch and the selected levels of takadiastase with 450 ml of water.

The results are recorded in Table VIII; the relation between diastatic activity of the starch preparations and the maximum viscosity of the pastes is shown in Figure 4. The wide differences in apparent

TABLE VIII

EFFECT OF ADDITIONS OF TAKADIASTASE UPON THE DIASTATIC ACTIVITY AND AMYLOGRAPH CURVE CHARACTERISTICS OF DIFFERENT WHEAT STARCHES

(Suspensions contained 9.1% starch)

Takadiastase added, %	Source of wheat starch				
	Durum wheat	Southwestern winter wheat	Hard red spring wheat	Minnesota winter wheat	Soft wheat
	DIASTATIC ACTIVITY, MALTOSE UNITS				
nil	4	3	10	2	2
0.042	42	31	27	12	10
0.210	111	78	66	35	34
0.525	184	131	104	62	60
	TEMPERATURE OF TRANSITION, °C				
nil	80.8	79.9	80.8	81.5	74.3
0.042	80.6	76.4	76.4	76.4	69.5
0.210	65.3	66.7	66.7	68.1	66.7
0.525	66.7	66.7	66.7	63.9	65.3
	PASTE TEMPERATURE AT MAXIMUM VISCOSITY, °C				
nil	94.5	94.8	95.0	95.0	91.2
0.042	94.5	94.5	94.5	93.1	92.4
0.210	93.1	91.7	93.8	93.8	90.3
0.525	93.1	70.7	69.5	68.1	68.1
	MAXIMUM VISCOSITY, BRABENDER UNITS				
nil	878	755	770	735	888
0.042	750	590	550	590	770
0.210	138	150	147	105	135
0.525	60	40	50	42	50

amyloclastic susceptibility of the various starches, as indicated by the variations in diastatic activity for corresponding increments of added takadiastase, may, in part at least, be due to varying degrees of mechanical injury of the starches during milling (Malloch, 1929; Karacsonyi and Bailey, 1930; Sandstedt, Blish, Mecham, and Bode, 1937; Sandstedt, Jolitz, and Blish, 1939; Jones, 1940; and others). The temperatures of transition and paste temperatures at peak viscosity

of the undiastated starches from the different hard wheats did not differ significantly, but these values were appreciably lower for the starch from the soft wheat. Diastating the starches resulted in a lowering of both the temperature of transition and the paste temperatures at peak viscosity, particularly for the two highest levels of

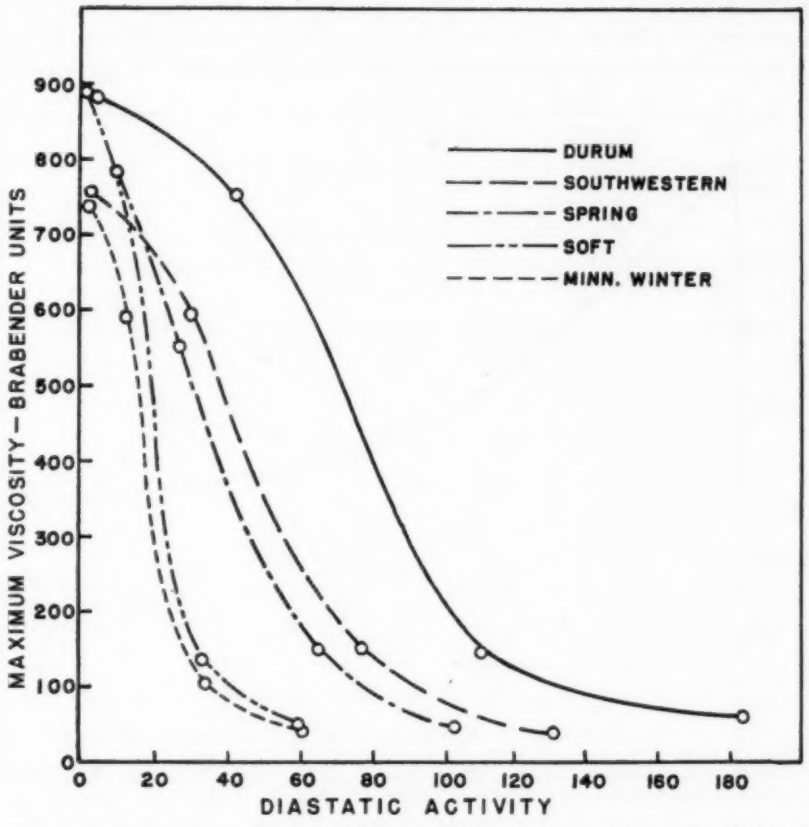

Fig. 4. Relation between diastatic activity and maximum paste viscosity for various wheat starch preparations. Diastatic activity was varied by additions of takadiastase to the starch. The starch suspensions contained 9.1% starch.

takadiastase. The maximum viscosity values for equal concentrations of the undiastated starches varied from 735 Brabender units for the Minnesota winter wheat starch to 888 units for the soft wheat starch. Increasing the diastatic activity markedly decreased maximum paste viscosity of each starch, but the relation between these variables is not strictly linear. Although the starches which showed the highest apparent amyloclastic susceptibility suffered the greatest decrease in viscosity, the maximum viscosity corresponding to a given maltose value varied widely with the different starches. For example,

a peak viscosity of 400 Brabender units was given by starches varying in maltose value from approximately 20 to 85 units. Accordingly, maximum paste viscosity cannot be interpreted as a direct index of amylase activity unless something is known about the maximum viscosity to be expected from the starch (or flour) in the absence of amylase activity.

Effect of Storage on Paste Viscosity of Commercial Wheat Starch. A marked decrease was observed in the paste viscosity of commercial

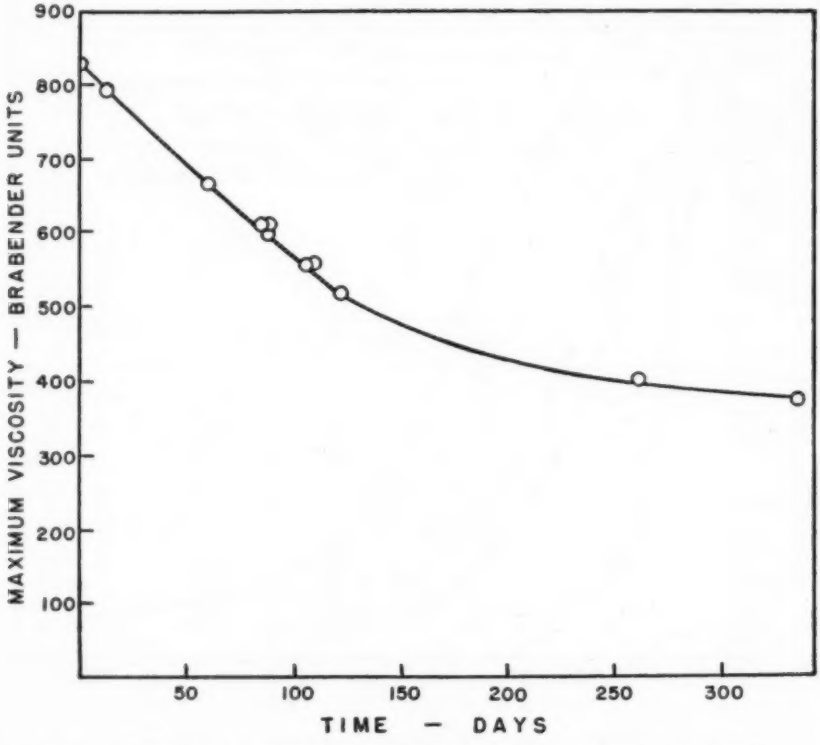

Fig. 5. Effect of time of storage of commercial wheat starch on maximum paste viscosity. Gelatinization tests were made with 9.1% suspensions.

wheat starch with time of storage. The starch contained 9.0% moisture (A. A. C. C. air-oven method) and was stored in glass bottles at laboratory temperature (22–25°C); the pH of a 10% suspension was 4.3, as determined with a glass electrode. Reitz, Gortner, and Carlson (1942) had previously noted the same phenomenon in the cold gelatinization behavior of air-dry wheat starch (10.6% moisture) which had been carefully prepared in the laboratory from Thatcher wheat.

The gelatinization behavior of the sample used in the present studies was followed in the amylograph at intervals over a period of

336 days, employing 50 g (dry basis) of starch and 450 ml of distilled water. As shown in Figure 5, the maximum viscosity dropped from an initial value of 830 B. U. to 370 B. U. after 336 days' storage. No consistent trends were observed in the temperature of transition or paste temperature at maximum viscosity. At the end of the storage period a portion of the starch was thoroughly washed with distilled water, centrifuged, and dried under vacuo at room temperature. This treatment was without any significant influence upon its gelatinization behavior. In view of these surprising results, time, and probably conditions of storage, are important factors to be taken into consideration in investigating the physicochemical properties of starches.

Effect of Cold Gelatinizing Agents on the Heat Gelatinization of Wheat Starch. Several reagents in aqueous solution cause starch to swell

TABLE IX

EFFECT OF COLD GELATINIZING AGENTS UPON THE HEAT GELATINIZATION BEHAVIOR OF COMMERCIAL WHEAT STARCH

Gelatinizing reagent	Reagent concentration	Temperature of transition	Paste temp. at max. vis.	Maximum viscosity
	M	°C	°C	*B. U.*
Control		70.9	90.3	557
Sodium salicylate	0.01	66.7	89.6	625
	0.025	67.4	88.4	720
	0.05	66.0	86.3	797
	0.10	63.9	83.4	972
Sodium thiocyanate	0.10	67.4	88.2	838
Potassium thiocyanate	0.10	66.7	88.1	847
Ammonium thiocyanate	0.10	67.4	88.2	837
Potassium iodide	0.10	66.7	89.6	785
Urea	0.10	67.4	90.0	576

or gelatinize at ordinary temperatures. Following the qualitative studies of Reychler (1920), a number of workers, including Ostwald and Frenkel (1927), Katz (1933), and numerous other papers, Mangels and Bailey (1933, 1933a), and Mangels (1934, 1936), have made quantitative studies of the relative efficiency of various cold gelatinizing agents and have applied them in investigating starches from various sources. Caesar (1932), Wiegel (1934, 1936), and Cook and Axtmayer (1937) have carried out heat gelatinization experiments in the presence of various reagents in which the viscosity of the starch suspensions was followed as the temperature of the suspensions was increased at a constant rate.

In the present study, six representative gelatinizing agents were employed, namely: sodium salicylate, sodium thiocyanate, potassium thiocyanate, ammonium thiocyanate, potassium iodide, and urea. The first four compounds hydrolyze to form an alkaline solution, potas-

sium iodide is a neutral electrolyte, and urea represents an organic swelling agent which forms a basic solution. Suspensions made with 50 g of commercial wheat starch and 450 ml of 0.1*M* solutions of each of these reagents were gelatinized in the amylograph over the usual temperature range of 25° to 95°C. In addition, curves were made with distilled water and three lower concentrations of sodium salicylate. The results are summarized in Table IX.

As compared with heat gelatinization in water alone, the cold gelatinizing agents decreased the transition temperature and the paste temperature at maximum viscosity but markedly increased paste viscosity. Sodium salicylate was the most, and urea the least, effective agent. The relative efficiencies of the salicylate, thiocyanate, and iodide ions are in the same order as that found by Mangels and Bailey (1933), who observed a lyotropic anion effect in their cold gelatinization studies.

TABLE X

EFFECT OF SODIUM SALICYLATE ON THE HEAT GELATINIZATION OF WHEAT AND POTATO STARCH [1]

Sodium salicylate conc.	Temperature of transition		Paste temperature at max. viscosity		Maximum viscosity	
	Wheat	Potato	Wheat	Potato	Wheat	Potato
M	°C	°C	°C	°C	*B. U.*	*B. U.*
0	85.2	64.2	94.3	81.0	20	470
0.1	58.6	62.8	67.0	72.6	60	340
0.2	53.0	58.6	62.8	67.0	135	400
0.5	51.6	47.4	69.1	55.1	330	590
0.6		42.5		50.2		700
0.8	33.4	34.1	48.8	41.8	475	770
1.0	27.1	28.5	32.7	36.2	540	890
1.2	25.0	25.0	26.4	33.4	420	990
1.4		25.0		26.4		940

[1] Amylograph curves were made with 30 g of starch and 450 ml of water or sodium salicylate solution.

In view of the marked effect of sodium salicylate on the heat gelatinization curve for wheat starch, additional curves were made with 30 g (dry basis) of commercial wheat and potato starch in 450 ml of various concentrations of this salt.

The mean results, summarized in Table X, show the very marked effects of sodium salicylate in lowering the temperature at which starch swelling becomes perceptible, in lowering the paste temperature at maximum viscosity, and in increasing the maximum viscosity. The greatest relative increase in viscosity was obtained with wheat starch and the maximum was registered at a lower concentration of sodium salicylate than with potato starch.

The markedly higher peak viscosities obtainable with cold gelatinizing agents, as compared with heat gelatinization, is of theoretical interest. Gortner (1933) applied the Kunitz formula to secure an estimate of the relative volumes of the disperse phase in cold and hot gelatinization of starch; the calculations indicated that the volume of the swollen granules was very much greater for cold gelatinization. This observation is in accord with the markedly lower temperature and higher peak viscosities found in this study when cold gelatinizing agents are present. These agents must greatly increase the ability of the starch granules to swell without granule disintegration taking

TABLE XI

EFFECT OF MODIFICATION ON CURVE CHARACTERISTICS FOR CORN STARCH

Sample No.	Corn starch	Temperature of transition		Paste temp. at max. viscosity		Maximum viscosity	
		Starch weight		Starch weight		Starch weight	
		35 g	50 g	35 g	50 g	30 g	50 g
		°C	°C	°C	°C	B. U.	B. U.
1	Unmodified	74.3		90.3		696	
2	Acid modified	74.3	70.9	90.0	85.8	138	422
3	Acid modified	73.0	70.9	87.6	82.4	120	405
4	Chlorinated	70.9	70.2	77.8	76.4	59	114
5	Chlorinated	68.1	66.4	73.0	72.5	54	85

place. In spite of extensive researches on the action of such agents, there is as yet no generally accepted theory which completely explains their behavior. Meyer (1942) has pointed out that starch grains have a limited capacity to swell in hot water and that such limited swelling is characteristic of chain polymers which are held together in large three-dimensional molecules by network secondary valence linkages which can be broken by chemically inert reagents. In starch, the swelling and solubilization of the amylose is limited by the lattice-like micelles formed by the amylopectin. If the secondary valence bonds acting between different parts of the amylopectin are broken, the micellar structure is opened up to form larger units thereby making it possible for the amylose molecules to take up more water and thus cause further swelling of the starch.

Effect of Processing Treatment of Curve Characteristics for Corn Starch. The characteristics of amylograph curves for five samples of commercial corn starch obtained from one source are summarized in Table XI. With one exception, two concentrations, namely 35 g and 50 g, of starch (dry basis) with 450 ml of distilled water, were employed. Starch No. 1 was a crude, unmodified or native starch; Nos. 2 and 3 were standard, acid-modified or thin-boiling starches representative of

the type used for warp-sizing in textile mills (No. 3 was the more highly modified); No. 4 was a chlorinated starch of the type used in sizing rayon, whereas No. 5 was a more highly modified chlorinated starch ordinarily used in the tub sizing of paper.

The relative curve characteristics for these starches are in line with the extent of their modification. Chlorination appreciably lowered the temperature of transition and the paste temperature at which maximum viscosity was attained, in addition to markedly decreasing the peak viscosity. The problem of evaluating starches prepared by various types and degrees of modification is a difficult one at present because they cover such a wide viscosity range that their relative viscosities cannot be satisfactorily determined by one method at one concentration. These limited trials suggest the possibility that relative values for the various types could be obtained in the amylograph because of the wide range of the instrument.

Discussion

These survey experiments show that the amylograph provides a convenient means for carrying out technological studies of the pasting properties of starches and of the effects of various agents on gelatinization characteristics. Because of the wide range in the magnitude of the viscosities which can be recorded and the various temperatures at which it can be operated, it may well prove useful in investigating the viscous or plastic properties of substances other than starches and flours. From the fact that the machine is calibrated in arbitrary units, and the rates of shear and of temperature increase are fixed, it is more useful in technological studies than as a research instrument.

The marked liquefying action of alpha-amylase on the viscosity of starch paste favors the use of the amylograph as a convenient means of determining the alpha-amylase activity of wheat and rye flours. However, such variables as pH, starch content, protein content, inherent differences in starch characteristics, and in the extent of mechanical injury suffered by the starch during milling influence paste viscosity and hence would interfere with the interpretation of relative height of the amylograph curve as a direct index of the alpha-amylase activity of flours which differ widely in these characteristics. In mill-control work, these interfering factors would not come into full play; the mill mix for any particular type of flour represents a composite of certain restricted types of wheat and the protein content is controlled within rather narrow limits. How closely maximum paste viscosity would be correlated with alpha-amylase activity under such limited conditions must be determined by further experiments.

Two difficulties were encountered which indicate that some im-

provement in the design of the instrument would be desirable. In certain of the experiments with cold gelatinizing agents, clot formation occurred around the upper part of the fixed center pin in the amylograph bowl. The other difficulty arose with very viscous pastes at the end of the heating period, especially when maximum paste viscosity occurred around 95°C. Under these conditions the contents of the bowl occasionally boiled over. The stirring was inadequate to maintain a uniform temperature throughout these viscous pastes, which resulted in the overheating and boiling over of the starch paste in the proximity of the outer edge of the bowl. It is interesting to note that Barham *et al* (1942) observed irregularities in viscosity at high concentrations during the heating period, with their rotating cylinder viscosimeter; these were ascribed to clot formation resulting from nonuniformity of the pastes.

Summary

Response of the amylograph to variations in the load applied to the viscosity-registering device was essentially linear. Wheat starch suspensions gelatinized in the amylograph from initial temperatures above 45°C gave markedly higher peak viscosities than corresponding suspensions gelatinized from lower initial temperatures. The precision of the measurement is satisfactory; for a series of 69 curves made in duplicate, the standard error (single determination) was 9.3 B. U.

With an increase in starch concentration, the temperature of transition and paste temperature at maximum viscosity decreased, the maximum paste viscosity increased, and the rate of decrease in viscosity after the maximum became greater. When the logarithm of the maximum viscosity was plotted against the logarithm of the starch concentration, a straight line resulted for all starches investigated (corn, potato, and wheat). This relation implies that starch swelling is a first-order process while granule disintegration is a second- or higher-order process.

Suspensions of wheat gluten and wheat starch gave higher paste viscosities than wheat starch suspensions of corresponding starch concentration.

Maximum paste viscosity of commercial wheat starch suspensions decreased in linear fashion with an increase in pH from 5.2 to 6.8. The decrease in viscosity was less with bimaleate than with citrate buffers.

Maximum paste viscosity of commercial wheat starch suspensions was markedly lowered when the starch was brought to increased maltose values by the addition of takadiastase (employed as a source of alpha-amylase).

Wheat starches prepared from durum, hard red spring, hard red winter, and soft winter wheat flours gave maximum paste viscosities for 9% suspensions which varied from 735 B. U. for Minnesota hard winter to 888 B. U. for soft winter wheat starch. Corresponding additions of takadiastase to these starches resulted in wide differences in paste viscosity. The relative maximum viscosity values corresponded, in general, to the apparent amyloclastic susceptibility of the starches as measured by maltose value. For any given paste viscosity, there was an appreciable range in the corresponding maltose value for the different starches.

Of several cold gelatinizing agents investigated with the amylograph, sodium salicylate was the most effective agent and urea the least effective. As compared with heat gelatinization in water alone, starch suspensions containing the more effective agents gave amylograph curves which were characterized by a lower transition temperature, lower paste temperature at maximum viscosity, and a markedly higher paste viscosity. Cold gelatinizing agents greatly increase the ability of the starch granules to swell without disintegration of the granules.

Wheat starch stored at 9.0% moisture at room temperature yielded suspensions of decreasing maximum paste viscosity with increased time of storage.

Marked differences were noted in the amylograph curve characteristics for native, acid-modified, and chlorinated corn starches.

The amylograph appears to provide a convenient means of investigating the pasting properties of starches from various sources, the relative resistance of different starches to amylases and other starch degrading agents, and the effects of different processing treatments.

Caution must be observed in interpreting the maximum paste viscosity of wheat- and rye-flour suspensions as an index of relative alpha-amylase activity of the flours because of the influence on paste viscosity of such variables as starch content, protein content, inherent differences between starches, extent of mechanical injury, and pH.

Acknowledgment

The authors are indebted to Arthur Hartkopf, Brabender Corporation, Rochelle Park, New Jersey, for the amylograph used in this study.

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THE USE OF THE AMYLOGRAPH IN THE CEREAL LABORATORY

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(Presented at the Annual Meeting, May 1943; received for publication May 19, 1943)

Numerous researches have been conducted on the utility of viscosity determinations in evaluating cereal products. In these investigations, many of which gave conflicting results, viscosity was usually measured at a fixed temperature, but lately several workers have studied the viscosity of cereal products under flexible, controlled temperature conditions. There is now available a recording viscosimeter, the Amylograph, which greatly facilitates studies of this nature, since viscosity can be measured and recorded automatically, either at a selected fixed temperature or under uniformly rising temperature conditions. Anker and Geddes (1944) [1] have described this instru-

[1] Anker, C. A., and Geddes, W. F., (1944) Gelatinization studies upon wheat and other starches with the Amylograph. Cereal Chem. 21: 335–360.

ment and have reported the results of investigations in technique, viscosities of different starches upon gelatinization, and effects of alpha-amylase on the gelatinization curves of starches. They also report the literature on the subject.

The experiments reported in this paper were undertaken to determine the value of the Amylograph in evaluating the baking quality of rye, for measuring the susceptibility of different flours to alpha-amylase, and as an index of the heat treatment of soybeans. The effect of such variables as granulation, pH, and electrolytes on paste viscosity were also investigated.

Experimental

Relation between Maximum Viscosity and Baking Value of Rye Meals. The baking quality of rye meals and flours is markedly influenced by their alpha-amylase activity. If this is too high, excessive liquefaction and dextrinization of the starch occurs, thereby lowering its ability to bind the water liberated by the denaturation of the proteins during baking. The bread from such flours and meals has a moist crumb with gummy characteristics. On the other hand, insufficient alpha-amylase is characterized by a dry, brittle crumb. Since these crumb properties are associated with the liquefying and dextrinifying action of alpha-amylase, a determination of the viscosity of gelatinized suspensions should provide a convenient means of evaluating rye products for baking.

One method for applying this test utilizes the MacMichael viscosimeter. A suspension of rye meal in distilled water at 30°C is heated to 75°C in 2.5 min at a uniform rate by heating the viscosimeter cup and contents in a water bath and using the spindle as a stirrer. The suspension is removed from the water bath and allowed to set for 2.5 min. The viscosity is then measured at 75°C.

The distribution of the viscosity data into the satisfactory and unsatisfactory classifications on the basis of baking tests is shown in Figure 1 for some 500 rye meals. The region where the two curves overlap is small, thus showing a definite correlation between gelatinized meal viscosity and baking quality under the specific test conditions employed.

The utility of the Amylograph for evaluating baking quality of rye meals was next investigated. For this purpose, 13 rye samples of varying baking quality were ground to such a granulation that 95% passed through a No. 16 wire sieve and 50% through a No. 30 wire sieve. A suspension containing 72 g of the meal (dry basis) in 400 ml of KH_2PO_4-NaOH buffer solution of pH 6.2 was placed in the Amylograph bowl, the machine started at 25°C, and the temperature allowed

to increase at the uniform rate of 1.4°C/min automatically provided by the instrument. The maximum viscosity was read from the curve in Brabender units (B.U.). The diastatic activity of the meals was measured as outlined in Cereal Laboratory Methods (4th ed., 1941). Rye muffin tests were used to evaluate baking quality.

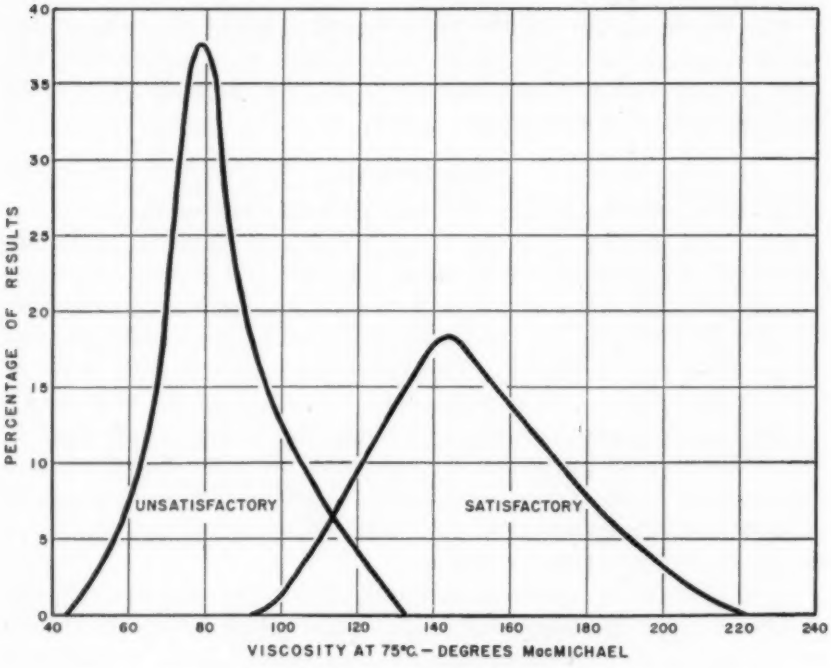

Fig. 1. Division of MacMichael viscosity data for rye meals into satisfactory and unsatisfactory values on basis of baking tests.

The results in Table I show that the higher the maximum viscosity, the better the baking quality, and the lower the diastatic activity. In other words, it is possible by means of the Amylograph to predict the baking quality and thereby furnish a basis for evaluating rye grain.

Effect of Starting Temperature, pH, Granulation, and Heat Treatment on Maximum Viscosity of Rye Meal Pastes. In testing rye products in the Amylograph, a period of about 22 min elapses before any increase in viscosity is registered. Different machine starting temperatures were used to ascertain whether this preliminary period could be reduced. The investigations were performed on rye meals using the same conditions as in the previous experiment.

Table II shows that the maximum viscosity is substantially unaltered until the starting temperature is raised above 40°C. Anker and Geddes (1944) [1] obtained a similar result in an analogous investigation

TABLE I

RELATION BETWEEN BAKING QUALITY, MAXIMUM VISCOSITY, AND DIASTATIC ACTIVITY OF RYE MEALS

Rye sample	Baking quality	Maximum viscosity	Diastatic activity mg maltose/10 g
		B.U.	
1	Very poor	150	540
2	Very poor	210	455
3	Poor	223	445
4	Fair	315	395
5	Poor	345	417
6	Fair	435	335
7	Good	442	365
8	Fair	445	382
9	Good	545	323
10	Good	547	352
11	Very good	763	275
12	Very good	1000+	268
13	Very good	1000+	85

with wheat starch, but the effect upon maximum viscosity of further increases in starting temperature was much less marked in their experiments. An increase of the starting temperature from 25°C to 40°C decreases the time required to make a curve on rye meal suspensions from 28 min to 12 min.

TABLE II

EFFECT OF STARTING TEMPERATURE ON MAXIMUM VISCOSITY OF RYE MEALS

Starting temperature	Rye meal No. 1		Rye meal No. 2	
	Max. viscosity	Time	Max. viscosity	Time
°C	*B.U.*	*min*	*B.U.*	*min*
25	150	28	545	28
30	155	24		
40	145	10	550	12
50	260	8	1000−	9
60	350	6		
70	375	5		

The effect of pH on maximum viscosity was investigated with suspensions of a corn starch and a medium rye flour brought to various pH values by means of KH_2PO_4-NaOH buffer mixtures. The results are presented in Figure 2. Decreasing the pH of the rye flour suspensions over the range studied markedly decreased the maximum paste viscosity; since there was no effect with corn starch this result must be attributed to increased alpha-amylase activity as the pH was decreased. The maximum viscosity of the rye flour paste at pH 7.0 was almost 250% greater than at pH 5.8; thus it is obviously necessary to control pH in evaluating rye flours by this method.

The effect of granulation on maximum viscosity was investigated by preparing rye meals of two granulations from one lot of rye. One sample of the grain was ground so that 95% passed through a No. 16 wire sieve and approximately 50% passed through a No. 30 wire sieve; the other sample was ground so that 95% passed through a No. 30

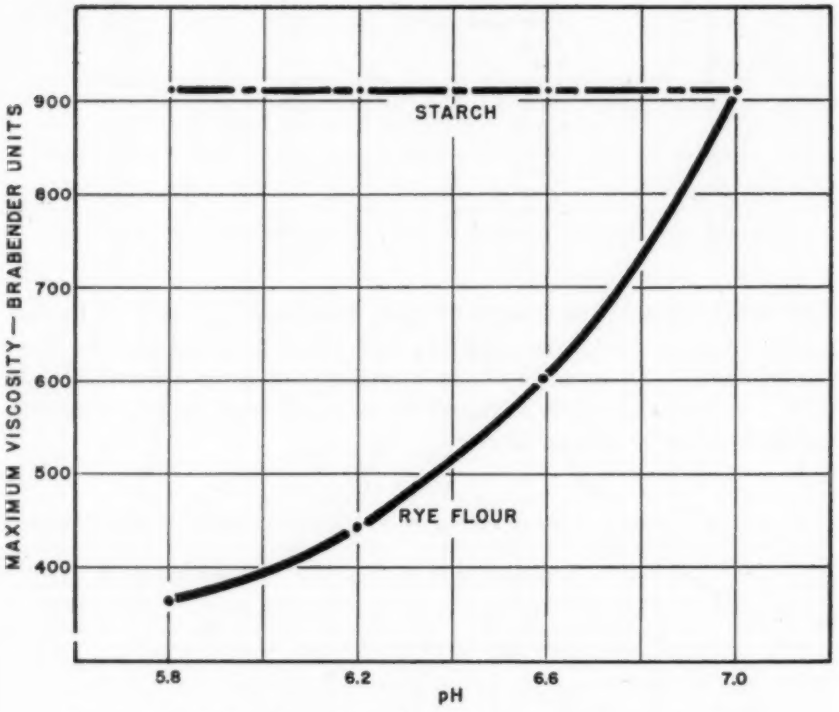

Fig. 2. Effect of pH on the maximum viscosity of a rye flour and a corn starch paste.

wire sieve. Amylographs were made for both meals, employing 72 g in 400 ml KH_2PO_4-NaOH buffer solution of pH 6.2 and a starting temperature of 25°C. The diastatic activity of each meal was also determined. The results were as follows:

	Coarse meal	Fine meal
Maximum paste viscosity, B.U.	320	535
Maltose value, mg/10 g	323	447

These data show the necessity of controlling granulation in evaluating different samples of rye by this test.

The marked effect of amylase activity on baking quality and maximum paste viscosity of rye meals was further shown by heating a sample of rye meal of poor baking quality in live steam at 120°C for

30 min. The results of diastatic activity, Amylograph, and baking tests for the control and the heated samples were:

	Control	Heat-treated
Maximum viscosity, B.U.	150	1000+
Maltose value, mg/10 g	540	85
Baking quality	Poor	Good

The higher maximum viscosity and better baking quality of the heat-treated sample are associated with a decrease in amylase activity due to heat inactivation of the enzyme.

Sensitivity and Replicability of the Amylograph. As a test of the sensitivity of the Amylograph, tests were made with corn starch suspensions in which the weight of starch was varied 2.5 and 5.0% above and below a reference weight of 40 g per 400 ml of buffer solution (KH_2PO_4-NaOH mixture) of pH 6.2. The results in Table III show

TABLE III

VARIATION OF MAXIMUM VISCOSITY AS RELATED TO VARIATION OF WEIGHT OF STARCH

Weight of starch		Maximum viscosity	
Grams	Variation	B.U.	Variation
	%		%
42	+5.0	990	+11.5
41	+2.5	935	+ 5.3
40	0	890	0
39	−2.5	820	− 7.6
38	−5.0	780	−12.2

that, on a percentage basis, the maximum viscosity values vary approximately twice as much as the variation in weight.

As a test of the precision with which the maximum viscosity of rye meal pastes could be determined, Amylograph tests were made in quintuplicate on 11 meals, employing the standard technique previously outlined. The results presented in Table IV show that the precision is quite satisfactory, especially when the magnitude of the viscosity values are taken into consideration.

Effect of Inorganic Salts on Maximum Paste Viscosity. As certain electrolytes are known to influence enzyme activity and also to affect starch paste viscosity, Amylograph curves were made with suspensions containing 72 g of a rye meal in 400 ml of 0.1% solutions of various salts at a pH of 6.2 (obtained with KH_2PO_4-NaOH buffer mixture).

The curves for those salts which influenced maximum paste viscosity are reproduced in Figure 3. The cupric, mercuric, cyanide, selenite, borate, and chromate ions increased, and calcium and stannous ions decreased, maximum paste viscosity. Other salts tried but which

TABLE IV

REPLICABILITY OF MAXIMUM VISCOSITIES ON RYE MEALS

Rye meal	Mean	Minimum	Maximum	Standard error (single determination)	Coefficient of variation
	B.U.	*B.U.*	*B.U.*	*B.U.*	%
1	150	145	155	3.5	2.3
2	223	200	235	14.0	6.3
3	210	200	220	10.0	4.8
4	315	305	330	11.7	3.7
5	345	325	375	20.9	6.0
6	435	425	470	19.7	4.5
7	442	425	470	17.2	3.9
8	445	430	460	11.2	2.5
9	545	515	580	25.5	4.7
10	547	510	580	28.6	5.2
11	763	735	800	27.3	3.6
All samples	402			18.8 [1]	4.7

[1] Computed from the results of a variance analysis of the data.

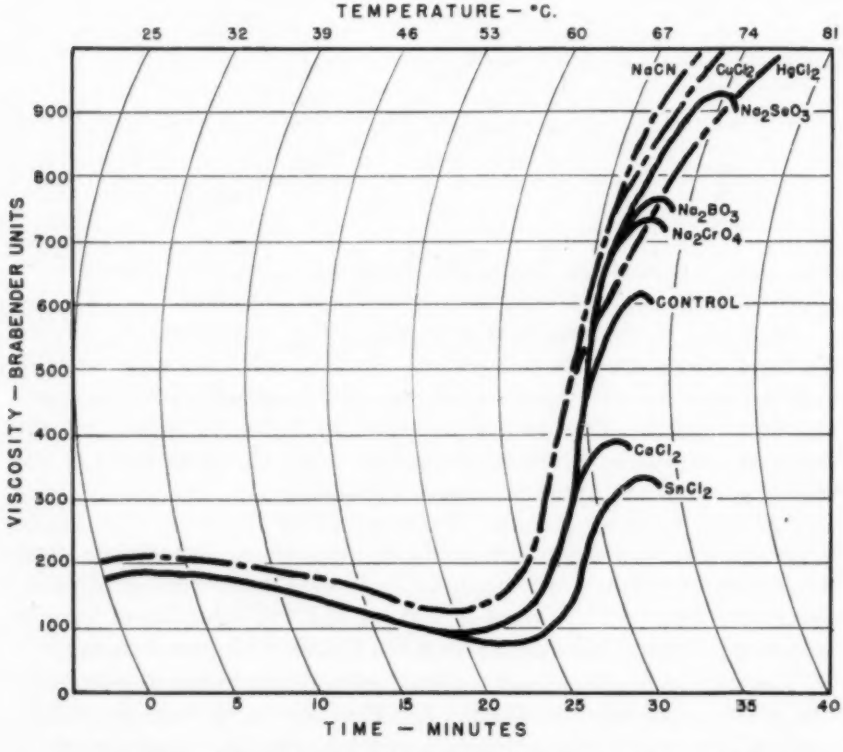

Fig. 3. Amylograph curves for suspensions of rye meal in 0.1% solutions of various salts. Buffer solution was used to obtain a pH of 6.2.

showed little or no influence on paste viscosity in 0.1% concentration were sodium chloride, barium chloride, ammonium chloride, ferric chloride, zinc chloride, potassium iodide, potassium bromate, sodium fluoride, lead nitrate, sodium arsenate, sodium tungstate, sodium citrate, sodium sulfate, and sodium nitrate.

Effect of Heat Treatment of Soya Meal on Maximum Viscosity. Since the heat processing of soya products is said to denature the proteins, thereby influencing their viscosity, experiments were con-

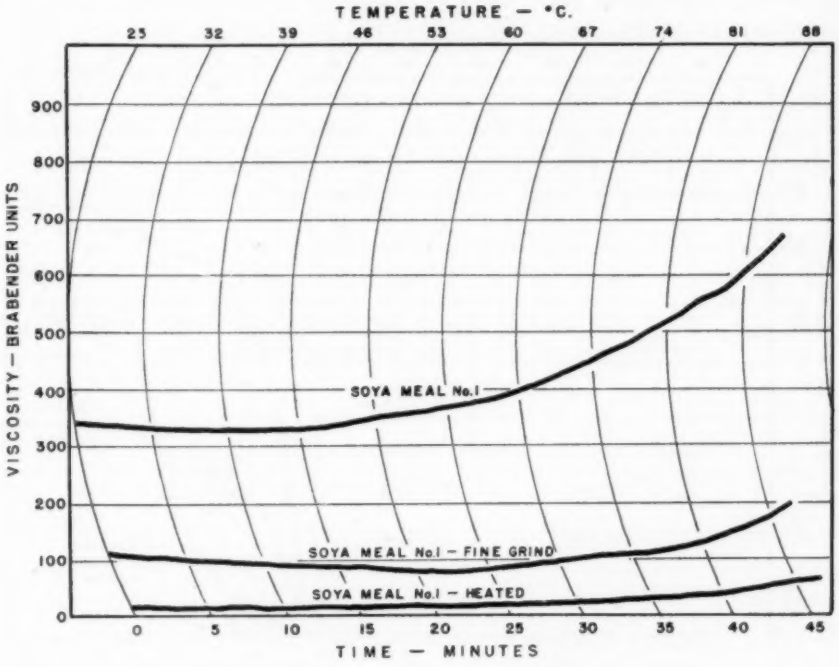

Fig. 4. Showing the effect of granulation and heat processing upon the Amylograph curve for soya meal.

ducted to determine the magnitude of this effect. A soya meal, ground to pass through a No. 18 wire sieve, was divided into three subsamples, one of which served as a control. The second subsample was processed in live steam at 120°C for 15 min, and the third was ground in a ball mill until it passed through a 13XX sieve. Amylograph curves were made by suspending 92 g of the meals in 450 ml of a KH_2PO_4-NaOH buffer solution at pH 6.2. A starting temperature of 25°C was used. Figure 4 shows that both fine grinding and heat processing lower the maximum viscosity. In certain chemically leavened products such as pancakes, it is necessary to employ soya products which give low viscosities.

Effect of Wheat Type and Amylase Activity on Maximum Paste Viscosity for Wheat Flours. Amylograph curves were made with a soft wheat flour, southwestern hard wheat flour, and a northwestern wheat flour which gave diastatic activity values of 112, 328, and 395 mg maltose/10 g of flour respectively. The first two flours were also diastated to increase their respective maltose values by 70 units. In making the curves, 45 g of the soft wheat flour, 63 g of the southwestern flour, and 77 g of the northwestern flour were respectively suspended in 400 ml of a KH_2PO_4-NaOH buffer at pH 6.2.

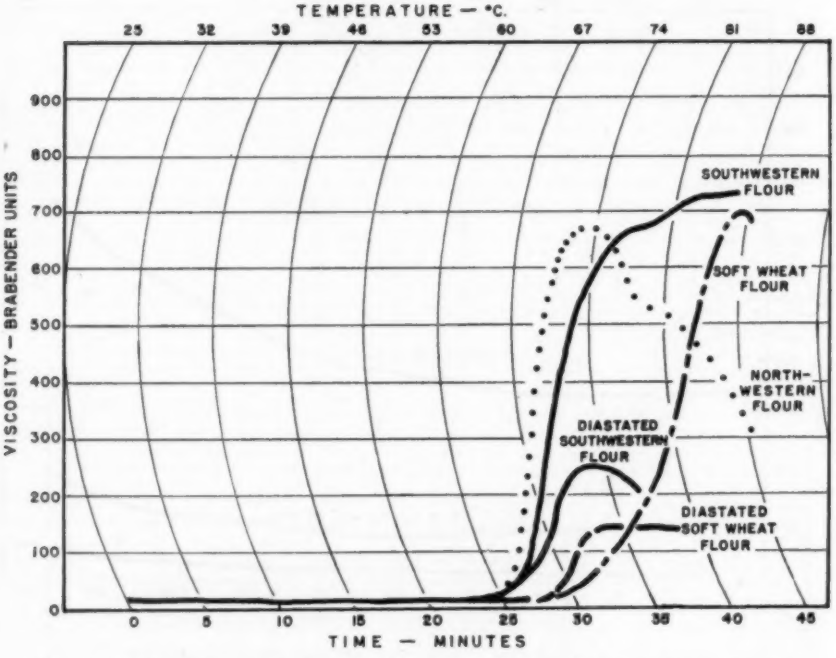

Fig. 5. Amylograph curves for different types of wheat flours. The effect of increasing the diastatic activity of two of the flours by 70 maltose units is shown.

The curves shown in Figure 5 illustrate the marked effect of diastating in decreasing the paste viscosity. They also indicate distinct differences between the amyloclastic susceptibility of the soft flour and southwestern hard wheat flour. Anker and Geddes (1944) [1] have carried out Amylograph studies with undiastated and diastated starches prepared from different classes of wheat. They found that the maximum paste viscosity for the undiastated starches differed materially and concluded that maximum paste viscosity cannot be interpreted as a direct index of amylase activity. The limited experiments reported here lend support to this view.

Summary

Maximum paste viscosity of rye meal suspensions, as measured by the Amylograph, can be employed to classify rye into groups which differ materially in baking value. The granulation of the meal and the pH of the suspension influence the viscosity values and must be controlled. With rye meals, the standard error of a single determination of maximum paste viscosity was 18.8 Brabender units. The time required for the test may be shortened by employing a starting temperature of 40°C rather than the customary temperature of 25°C. Starting temperatures above 40°C give increased paste viscosity values.

In addition to evaluating rye samples, the Amylograph is well adapted for investigations of the effect of inorganic salts on paste viscosity, for determining the effect of granulation and heat processing on the viscosity of soya products, and for viscosity studies on wheat flours.

A NOTE ON THE PRESENCE OF FIBER IN THE "AMYLODEXTRIN" FRACTION OF WHEAT FLOUR

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(Received for publication January 3, 1944)

In the course of experiments on starch manufacture, starch suspensions obtained by washing gluten from flour-water doughs were strained through silk 8XX. There remained on the silk a small quantity of a whitish sludge which obviously was not starch. Microscopic examination revealed that it consisted mainly of fragments of thin cell walls with considerable bran powder and some starch embedded in the mass. As this material showed a certain similarity to the "amylodextrin" fraction described by Sandstedt, Jolitz, and Blish (1939),[1] the top layer of a centrifuged starch suspension was examined microscopically. It was found to consist of the same kind of cell wall fragments that made up the sludge, but it contained more starch, and for this reason the bran powder was more dilute. The starch granules embedded in this top layer were of much smaller average size than those found in the bottom layer.

Owing to the removal of the sludge, the volume of the top layer decreased considerably when the starch suspension was strained

[1] Sandstedt, R. M., Jolitz, C. E., and Blish, M. J. Starch in relation to some baking properties of flour. Cereal Chem. 16: 780–792, 1939.

through fine bolting silk previous to centrifuging. By repeated straining through bolting silk of increasing fineness, 5XX, 8XX, 10XX, practically all the cell wall fragments could be removed. On centrifuging the strained starch suspension, a top layer of much smaller volume than that of the original one was obtained, but now it consisted almost exclusively of small starch granules. Staining with 0.2 solution of congo red greatly helped to distinguish the cell wall fragments on the slide.

These findings suggested that the unidentified matter referred to by Sandstedt *et al* is cellulose. To verify this assumption, crude-fiber determinations were made on a flour and on the starch and "amylodextrin" layer obtained from it. The results, on a 12% moisture basis, were flour 0.34%, starch 0.19%, and amylodextrin 0.85%. As the flour yielded 58% starch and 18% amylodextrin, 0.11 g of the total fiber is present in the starch, and 0.15 g in the "amylodextrin" fraction.

The observations and figures reported in this note show that the "amylodextrin" layer cannot be regarded as a distinct chemical fraction. Sandstedt *et al* allowed for this possibility by using the term "amylodextrin" only tentatively.

SOURCES OF ERROR IN THE DETERMINATION OF THE PROTEIN CONTENT OF BULK WHEAT

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(Received for publication April 28, 1944: presented at the annual meeting, May 1944)

From the time of sampling a car of wheat until the analysis is completed, there are numerous actual or potential sources of error which contribute to lack of agreement between protein determinations made on the same wheat by different laboratories. The present study was designed to afford an estimate of the relative importance of these several sources under normal working conditions. The factors studied were (1) heterogeneity of bulk wheat; (2) sampling error; (3) variation in cleaning procedure; (4) differences in grinding methods; and (5) analytical error.

Experimental

Heterogeneity of Wheat. Because individual wheat kernels from the same sample may differ widely in protein content as in other properties, it is impossible to take two identical subsamples of reason-

able size from a single bulk lot of grain, no matter how careful the blending and sampling may be. In an attempt to estimate the magnitude of this variation, 16 samples of clean wheat were thoroughly blended in a MacLellan mixer and divided into two equal portions by means of a Boerner sampler. One of the subsamples was ground in its entirety, blended, and analyzed in triplicate. The second subsample was divided into three equal portions and each portion was then ground and analyzed. The mean squares for the errors (within samples) of the two procedures were found to be 0.0042 and 0.0103 respectively. If it is assumed that grinding and blending the entire subsample produced a completely homogeneous material, then the value 0.0042 represents analytical variation alone, and the difference between the two mean squares (0.0103 − 0.0042), or 0.0061, is a measure of variability in clean, carefully blended wheat. This gives a standard error of 0.078% protein. In other words, if a series of samples were withdrawn from a bulk lot of clean wheat and were then analyzed with perfect accuracy, the results would still be scattered in such fashion that about one third of the values would vary from the mean by more than 0.078% protein.

Sampling Error. In addition to the error resulting from variation in wheat, there may be some variation arising from differences in technique when two operators sample the same car or from the fact that bulk lots may not be thoroughly mixed. To estimate the importance of this source of error, 16 cars representing random commercial shipments were sampled by two operators, each using his customary procedure. The duplicate samples were then cleaned and analyzed in triplicate.

As would be expected, the data revealed no systematic difference between operators. However, an appreciable random variation between pairs of samples was found. When other sources of error were accounted for, it was found that the mean square for differences in sampling technique and heterogeneity of wheat amounted to 0.0417. Assuming that the value found for variation in wheat alone also applies in this case, the mean square for sampling amounts to 0.0417 − 0.0061, or 0.0356. The corresponding standard error is then 0.189% protein.

Cleaning Procedure. All laboratories do not use the same kind of equipment for cleaning wheat samples prior to analysis, and the amount of small kernel wheat, weed seeds, and the like which is removed may have an appreciable bearing on the final protein content. An experimental determination of the importance of variation in cleaning techniques was made by carefully blending 16 uncleaned wheat samples, dividing them on a Boerner sampler, and cleaning the subsamples, in each of two laboratories, by the procedures customarily

employed. The cleaned samples were then reduced, ground, and analyzed in the same laboratory. After the effect of other sources of variation was removed by appropriate analysis, it was found that the standard error of a single determination for this source of variability was 0.182% protein.

Grinding Methods. It was assumed that different laboratories do not employ identical procedures in the grinding of whole wheat samples prior to analysis. The differences in practice may well contribute to variability in results. To measure the magnitude of this source of error, eight clean wheat samples were carefully blended, subdivided by means of the Boerner divider, and sent to each of 10 laboratories to be ground and analyzed. Information was available on the magnitude of analytical error among these laboratories. It was assumed that, after deducting variation between samples, the residual scattering was due to three sources, namely, heterogeneity of wheat, analytical error, and variations in grinding technique. Since the first two factors had been evaluated, the error arising from their operation was removed from the total, leaving a standard error for grinding of 0.165%.

Analytical Error. All laboratories will not agree exactly when determining the protein content of a completely homogeneous sample. Data were available for the interlaboratory variation of the 10 laboratories mentioned in the preceding paragraph from collaborative analysis of eight well-blended flour samples. It has been assumed that these samples were entirely uniform and that any variation in results would be due to differences in analytical technique alone. The standard error of a single determination for this source of difference between laboratories was found to be 0.110% protein. This value agrees well with the estimate of interlaboratory error, 0.10%, found by Davis and Wise (1933) and for the intralaboratory variation, also 0.10%, reported by Geddes and Milton (1939).

Discussion

The findings discussed above are summarized in Table I. Of the five sources of error investigated, it is apparent that differences in sampling, cleaning, and grinding technique are the most important in contributing to variation in wheat protein results. The variation ascribed to heterogeneity of wheat of necessity represents the irreducible minimum of scatter which would be obtained if all other errors were eliminated. Of the four remaining sources of variation, cleaning technique and grinding procedures seem to offer the greatest opportunity for improvement. The equipment can be better standardized

and procedures more carefully controlled. Sampling techniques are fairly well established, and it seems probable that the variation found is due not so much to technique as to lack of uniformity in the bulk wheat.

TABLE I

INTERLABORATORY ERRORS IN WHEAT PROTEIN DETERMINATIONS

Source of error	Standard error of single determination
	%
Heterogeneity of wheat	0.078
Sampling	0.189
Cleaning	0.159
Grinding	0.165
Analysis	0.110
All sources combined	0.307

The various sources of error are not additive in the form of standard errors. However, they may legitimately be added as squares of these errors. When this is done and the square root of the sum extracted, the over-all standard error is found to be 0.307%. Thus if a car of wheat is sampled by two individuals and the samples are cleaned, ground, and analyzed by a single determination in two laboratories, the results will differ by more than 0.31% about one third of the time. This agreement will not be substantially improved by carrying out duplicate determinations on the ground sample because the analytical error is a relatively small portion of the total. It can be estimated that the standard error will be reduced only to 0.297% protein.

As with all evaluations of this kind, the results listed above have an element of uncertainty inasmuch as the study was carried out with a limited number of samples. The values obtained for error due to differences in grinding technique and to analytical variation are probably more generally reliable than are the other factors since they were calculated from data accumulated by 10 laboratories in contrast to the two laboratories involved in the measurement of sampling and cleaning errors. It is believed, however, that the estimates are reasonably accurate, for the over-all error agrees well with that found in actual practice. Data were available on the analysis of 158 cars of wheat by the two laboratories whose data were used to evaluate sampling and cleaning errors. These laboratories were also included in the studies designed to estimate the other errors. The over-all variation for these 158 pairs of determinations was found to give a standard error of 0.29%, a value in good agreement with the figure of 0.297% calculated from the laboratory studies.

Summary

Interlaboratory variation in determining the protein content of bulk wheat is caused chiefly by differences between replicate samples. Differences in technique in preparing samples for analysis (cleaning and grinding) are somewhat less important causes, while analytical error is relatively small. Accordingly, little is gained in precision by carrying out replicated analyses of the same subsample of ground wheat. Improvement in results should rather be sought in better sampling and cleaning techniques and in a more uniform grinding procedure.

Acknowledgments

For their active and generous assistance in carrying out the work reported in this paper we are indebted to Mr. W. R. Young and Mr. John Dwyer of the Minnesota State Grain Inspection Department; and to Messrs. F. A. Collatz, E. V. Hetherington, C. L. Moore, George Swindaman, W. M. Tinkham, E. F. Tibbling, A. A. Towner, B. H. Kepner, A. C. Conner and L. Reimers of General Mills, Inc.

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THE EFFECT OF TEMPERATURE DIFFERENCES ON SOME MIXOGRAM PROPERTIES OF HARD RED SPRING WHEAT FLOURS[1]

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(Received for publication January 21, 1944)

The effects of temperature variations on the physical properties of dough have been described by a number of workers. Harrel (1927), when determining the stiffness or toughness of doughs with a gravimetric penetrometer, observed that the viscosity decreased with increasing temperature. Skovholt and Bailey (1932) demonstrated an increase of mobility with temperature rise, the variations amounting to 12 to 40 Farinograph units per degree C, depending on the stiffness of the doughs.

Bohn and Bailey (1936) observed the effect of raising the temperature in reducing Farinograph and stress readings. The latter were reduced approximately one third. Halton and Scott Blair (1937)

[1] Published with the approval of the Director of the Station.

found the viscosity of doughs, as ascertained by the stress extensimeter, to fall about 10% for each degree C rise of temperature. Stamberg and Bailey (1940) pointed out the need for accurate temperature control when measuring dough plasticity with a plastometer, as small temperature variations affected the rate of flow to a greater extent than any probable pressure differences. Moore and Herman (1942) studied the effect of temperature on three Farinograph properties. These were the initial phase, period of resistance, and a factor X calculated from these. As temperature increased, the initial phase and X decreased, while the period of resistance lengthened.

As increasing attention has been given in this laboratory to the estimation of certain properties of mixograms as supplementary criteria of baking quality, it was thought advisable to inquire into the influence of mixing temperature on these properties.

Mixograms were made over a temperature range of 35°C with four hard red spring wheat flours adjusted to two protein levels. In addition, the influence of temperature differences on curve pattern was compared with the effects produced by varying the absorption.

Materials and Methods

Four long-patent flours experimentally milled from hard red spring wheats of the 1942 crop were employed in the study. These wheats consisted of one sample each of Thatcher, Rival, Vesta, and No. 2822 and were free from visible forms of damage. Comparative analytical data for the four wheats and the resultant flours are shown in Table I.

TABLE I

COMPARATIVE ANALYTICAL DATA FOR THE FOUR HARD RED SPRING WHEATS AND LONG-PATENT FLOURS

Variety[1]	Test weight	Wheat protein[2]	Flour yield	Flour ash[2]	Flour protein[2]	Absorption	Loaf volume
	lb/bu	%	%	%	%	%	*cc*
Thatcher	62.3	13.9	71.4	0.41	12.9	62.1	665
Vesta	63.7	13.9	75.6	0.45	12.8	61.8	680
No. 2822	61.7	14.9	74.0	0.44	14.1	62.8	700
Rival	63.2	14.4	70.6	0.50	13.2	62.8	735

[1] All samples graded 1 heavy dark northern spring.
[2] Expressed on 13.5% moisture basis.

Characteristic mixograms of these varieties at the original flour protein content are shown in Figure 1. Wheat No. 2822 is a new unnamed variety with a short dough development and dough stability time, but generally has high protein content and good loaf volume. Vesta, on the other hand, tends to have an exceptionally long dough development period and is relatively resistant to overmixing. Thatcher and

Rival are somewhat similar in their comparative mixing patterns and could be considered approximately average for hard red spring wheat varieties.

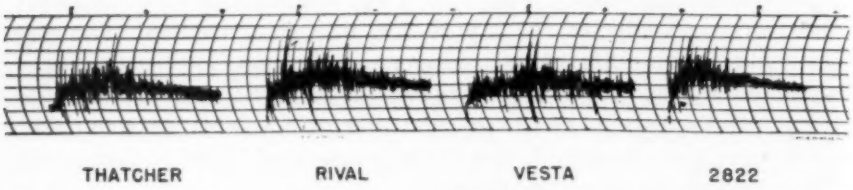

Fig. 1. Comparative mixograms for flours representing the four varieties at the original protein level, using a temperature of 30°C.

The four flours were each diluted to uniform protein levels of 10% and 12% with the same sample of experimentally prepared wheat starch.

In studying the effect of temperature differences on curve pattern, mixograms were made with two formulas: one, a flour-water mix, and the other, flour with the ingredients of the malt-phosphate-bromate formula. Twenty-five g of flour was used throughout. A constant absorption, as determined when baking the samples, was employed for each flour. Mixograms were made at eight temperatures which covered a range of 35°C. It was found convenient to make one determination at the low level of 5°C as a room was available which is held at this temperature. The mixograph, with the cabinet completely opened up, and accessory equipment was placed in the room for some time before the experiment was started, and the work was performed in it. The other temperatures were obtained by a combination of room and mixograph cabinet control. A Bahnson laboratory humidifier was installed in the mixograph cabinet to maintain the relative humidity at approximately 75% throughout the experiment. Temperatures were checked throughout the experiment and care was taken to warm or cool mixograph bowls, flours, and solutions to the required point before mixing.

To determine whether changes in flour absorption would have the same effect on curve pattern as differences in temperature, mixograms were made at 30°C for one flour, employing the baking formula at absorptions of 56, 62, and 68%.

The particular mixogram properties selected for measurement were dough development stage, curve height, and curve width. Dough development stage and curve height have been described by Harris (1943) with methods of estimation. The first depends upon the horizontal distance from the commencement of mixing to the point corresponding to the curve peak or point of optimum development, while

curve height is denoted by the length of the line drawn through the peak point to the edge of the chart paper. The width was estimated from the curve at the point corresponding to maximum height.

Experimental Results

Effect of Variations in Temperature on Mixogram Pattern. The mixograms obtained with the four flours adjusted to a protein level of

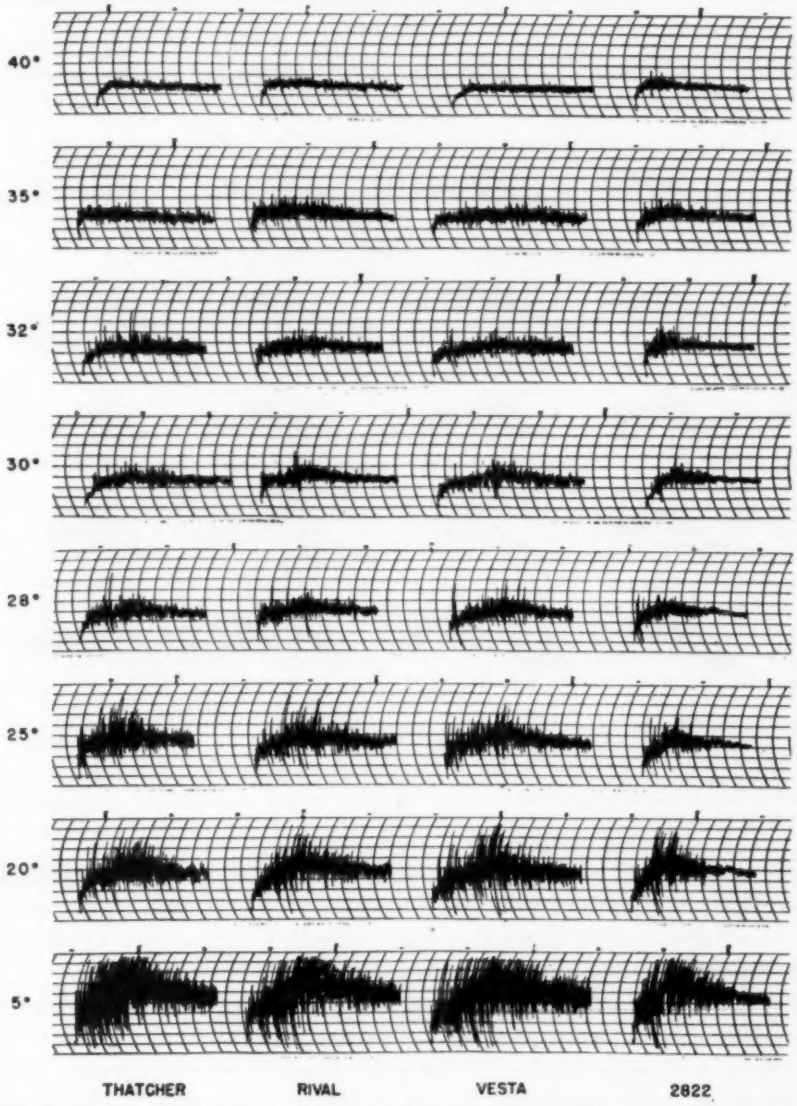

Fig. 2. Effect of mixing temperature on the mixogram patterns for flours representing four hard red spring wheat varieties. The flours were diluted with wheat starch to a constant protein level of 12.0%. Mixograms were made with flour and water only.

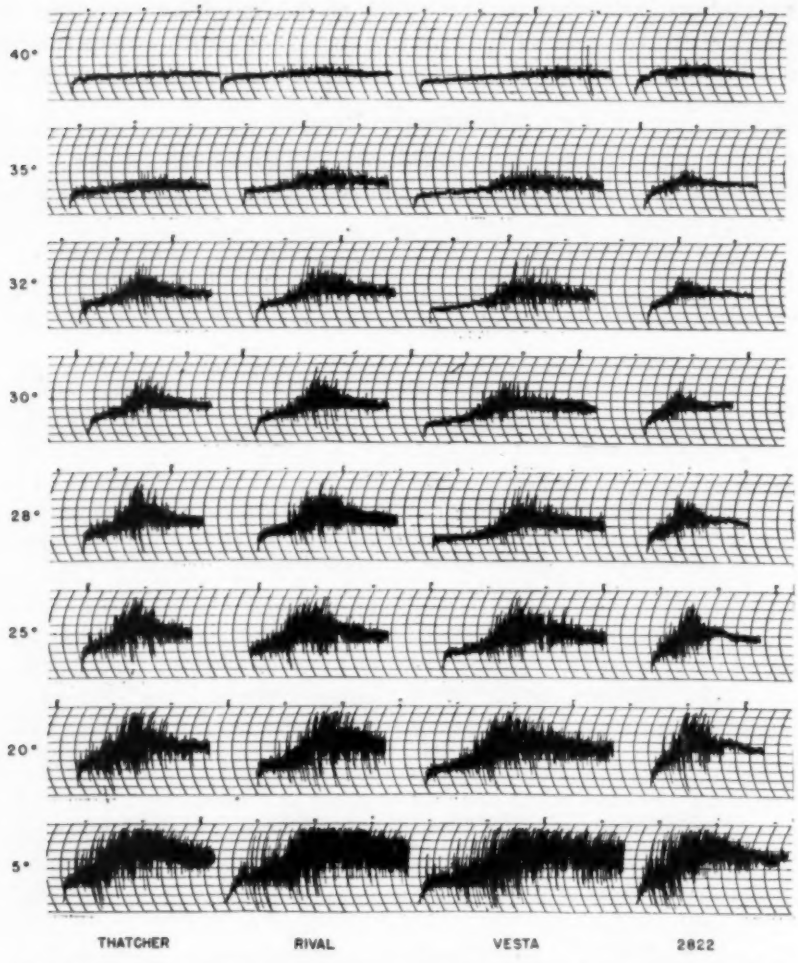

Fig. 3. Effect of mixing temperature on the mixogram patterns for flours representing four hard red spring wheat varieties. The flours were diluted with wheat starch to a constant protein level of 12.0%. Mixograms were made with flour and the ingredients of the malt-phosphate-bromate baking formula.

12% are reproduced in Figures 2 and 3. The mean values for dough development stage, curve height, and curve width for each temperature and each flour (variety) are shown in Table II, together with a variance analysis of the data.

For all three curve properties except dough development stage for the baking-formula doughs, the values markedly decrease with an increase in the temperature at which the mixograms were made. The decrease is fairly consistent for curve height and width for both the formulas and is substantially greater for curve height. With the bak-

ing-formula doughs, the initial decrease in dough development with an increase in temperature is followed by a sharp increase at temperatures above 30°C; this is due to the exceptionally long gradual rise in curve height to the peak. For all curve characteristics, a difference of 5°C

TABLE II

MEAN VALUES OF SOME MIXOGRAM PROPERTIES AS INFLUENCED BY TEMPERATURE DIFFERENCES AND WHEAT VARIETY

FLOURS ADJUSTED TO A UNIFORM PROTEIN CONTENT OF 12.0%

Temperature	Dough development stage		Curve height		Curve width	
	Flour-water mixes	Baking[1] formula mixes	Flour-water mixes	Baking[1] formula mixes	Flour-water mixes	Baking[1] formula mixes
°C	cm	cm	cm	cm	cm	cm
40	2.6	13.0	5.3	5.6	0.7	0.9
35	4.9	11.1	5.9	6.5	1.2	1.3
32	5.5	8.9	6.3	7.4	1.2	1.6
30	6.0	8.3	6.7	7.7	1.0	1.7
28	5.5	8.5	6.8	8.0	1.4	1.8
25	5.6	7.7	7.6	8.6	1.8	2.2
20	5.9	8.7	8.2	9.2	2.4	2.0
5	7.2	11.7	10.2	10.6	3.3	2.4
WHEAT VARIETY MEANS						
Vesta	6.9	13.3	7.0	7.7	1.7	1.8
Rival	5.4	10.4	7.2	8.2	1.6	1.9
Thatcher	5.5	9.5	7.1	7.9	1.7	1.7
No. 2822	3.7	5.8	7.2	7.9	1.5	1.6

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Variances[2]		
		Dough development stage	Curve height	Curve width
Temperatures	7	6.09**	18.83**	3.29**
Varieties	3	77.51**	0.29**	0.15
Mixes	1	301.46**	10.16**	0.17
Interaction:				
Varieties × mixes	3	13.03**	0.05	0.11
Temperatures × varieties	21	0.91	0.09*	0.12*
Temperatures × mixes	7	15.67**	0.24**	0.54**
Temperatures × varieties × mixes	21	1.57	0.04	0.05
Total	63			

[1] The baking formula consisted of the following ingredients in the specified percentages: high diastatic malt 0.3%; ammonium di-hydrogen phosphate 0.1%; potassium bromate 0.001%; sucrose 5.0%; sodium chloride 1.0%; yeast 3.0%.

[2] The interaction, temperatures × varieties × mixes, was used as error.

* Denotes significance exceeding 5% point.

** Denotes significance exceeding 1% point.

causes distinct changes and even a variation of ±2°C from 30°C produces noticeable differences.

The long dough development period of Vesta and the extremely short development time of No. 2822 are in agreement with the previous observations of Harris, Sibbitt, and Elledge (1944). Varietal differ-

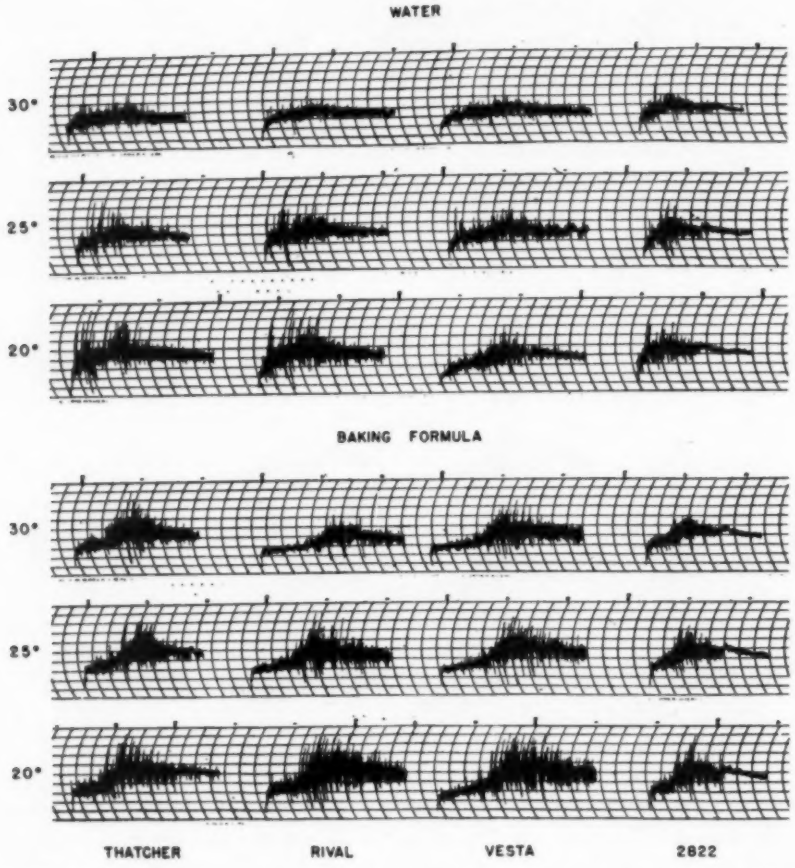

Fig. 4. Effect of mixing temperature on the mixogram patterns for flours representing four hard red spring wheat varieties. The flours were diluted with wheat starch to a protein level of 10.0%.

ences are largely obscured when the curves are made at temperatures which deviate markedly from the normal. Thus at 40°C the mobility of the dough is so great that very little resistance is offered to mixing and the curve is low and extremely narrow; whereas at 5°C the curve is high and very wide. These changes render it difficult to detect differences due to variety. It is apparent that varietal differences are more pronounced when the flours are mixed with the ingredients of the baking formula than when water alone is used. The variance analyses

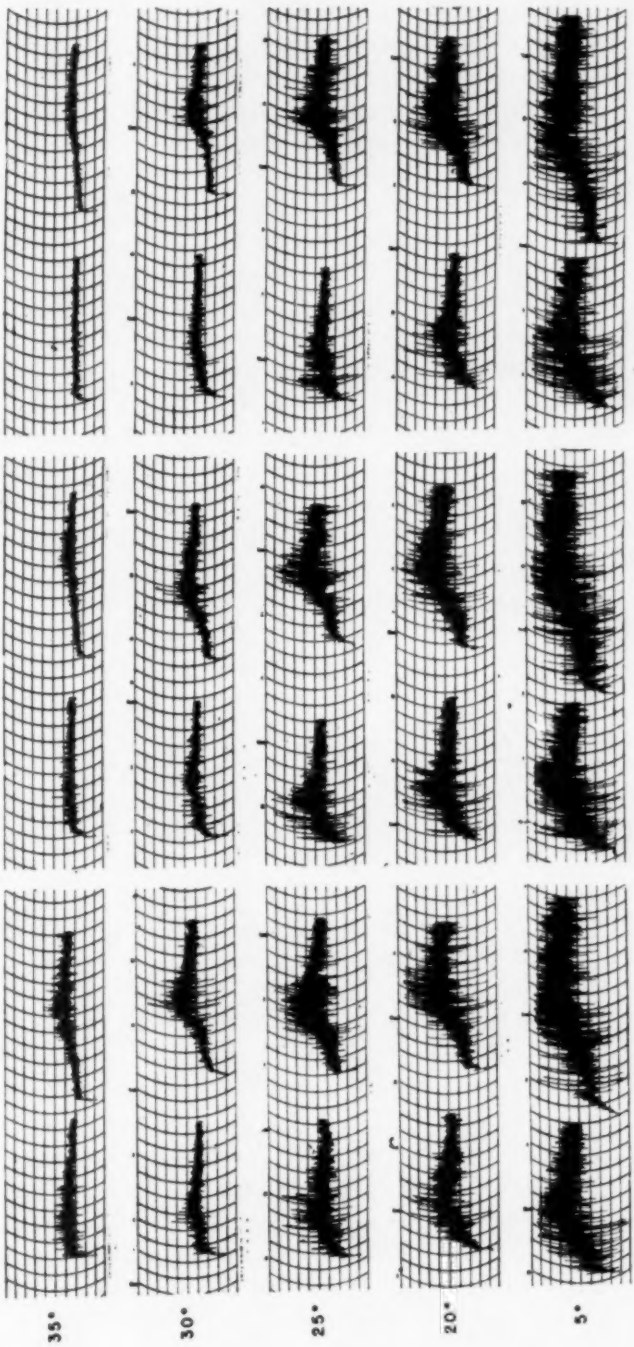

Fig. 5. Effect of flour protein content on mixogram patterns. A hard red spring wheat flour was diluted with wheat starch to produce the three protein levels.

show that the effect of temperature on curve height and width is not uniform for the different varieties. The dough development stage, however, varies with temperature in essentially the same manner for all the varieties. Moreover, the precise effect of temperature differs, depending upon whether the doughs are mixed with water or with the ingredients of the baking formula.

Mixographs for the four flours adjusted to the comparatively low protein level of 10% are shown in Figure 4 for temperatures of 20°, 25°, and 30°C. The flour-water curves made at 30°C are quite useless for differentiating between the varieties; for this purpose, those obtained at 20°C are probably the most valuable. With the baking-formula doughs, the authors prefer the mixograms secured at 25°C for varietal differentiation.

In addition, as shown in Figure 5, curves were made with both formulas, at temperatures of 5, 20, 25, 30, and 35°C, employing a hard red spring wheat flour adjusted to protein levels of 10, 11, and 12%. Mean values for the mixogram characteristics for each temperature and protein level are recorded in Table III, together with a variance analysis of the data. The principal additional point of interest in these studies is that the protein level had less effect on curve properties than the variations in temperature which were used. Curve height was the only mixogram characteristic significantly influenced by protein content. As protein content was decreased, the curve height became less. This is also evident from a comparison of the curves given in Figure 4 with those of Figures 2 and 3. Swanson (1941) found that lowering the flour protein reduced curve height but did not affect the varietal pattern of hard red winter wheat flours.

These data show the very marked influence of mixing temperature upon the mixogram pattern and emphasize the importance of close temperature control in mixogram studies.

The Comparative Effects of Variations in Temperature and Absorption on Mixogram Properties. Curves made at 30°C with a hard red spring wheat flour and the ingredients of the baking formula employing three absorptions are shown in Figure 6. The normal baking absorption for this flour was 62%. The dough mixed at 68% absorption was very slack, while that mixed at 56% absorption was decidedly stiff. While an increase in absorption, like an increase in mixing temperature, decreased curve height and, to a lesser extent, curve width, the dough development stage was lengthened. The influence of a temperature change on curve pattern is therefore not the same as the effects produced by varying the flour absorption.

TABLE III

MEAN VALUES OF SOME MIXOGRAM PROPERTIES AS INFLUENCED BY TEMPERATURE DIFFERENCES AND PROTEIN LEVEL

Temperature	Dough development stage		Curve height		Curve width	
	Flour-water mixes	Baking[1] formula mixes	Flour-water mixes	Baking[1] formula mixes	Flour-water mixes	Baking[1] formula mixes
°C	*cm*	*cm*	*cm*	*cm*	*cm*	*cm*
35	6.3	11.8	5.8	6.2	0.9	1.2
30	6.5	9.5	6.5	7.3	1.1	1.5
25	5.6	8.2	7.6	8.3	1.8	2.2
20	6.1	9.2	8.0	8.9	2.1	2.4
5	8.1	13.4	9.7	10.1	3.0	2.7
EFFECT OF PROTEIN LEVEL						
Protein level, %						
12	6.0	10.2	7.8	8.7	1.8	2.0
11	6.5	10.5	7.7	8.1	1.8	2.1
10	7.2	10.5	7.0	7.6	1.7	2.0

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Variances[2]		
		Dough development stage	Curve height	Curve width
Temperatures	4	13.22**	13.64**	3.02**
Protein levels	2	1.42	2.57**	0.03
Mixes	1	113.29**	3.14**	0.36
Interaction:				
Temperatures × mixes	4	2.67*	0.09	0.11
Protein levels × temperatures	8	1.45	0.06	0.15
Protein levels × mixes	2	0.63	0.15*	0.02
Protein levels × temperatures × mixes	8	0.69	0.04	0.13
Total	29			

[1] See Table II footnote 1 for description of baking formula.
[2] The interaction, temperature × varieties × mixes, was used as error.
* Denotes significance exceeding 5% point.
** Denotes significance exceeding 1% point.

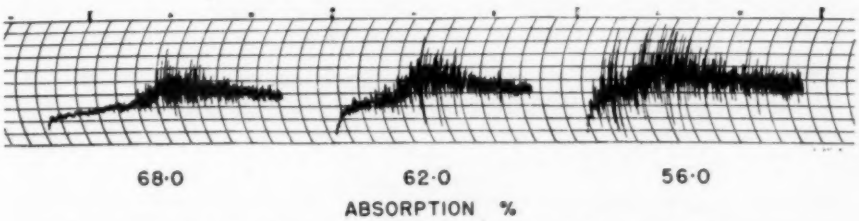

Fig. 6. Effect of absorption on curve pattern at a protein level of 12.0%. Mixograms were made at 30°C with the ingredients of the malt-phosphate-bromate baking formula.

Discussion

The marked changes in curve pattern as the temperature is increased may be attributed to (1) a decrease in the viscosity of the interstitial water (free water); (2) a decrease in the amount of adsorbed (bound) water, and (3) a decrease in the intensity, or in the relative number of the forces of interaction functioning between the nonwater dough components, particularly the proteins. The relative importance of these factors cannot be separately evaluated. The fact that there is a differential reaction of the varieties to the effect of temperature on curve pattern indicates that the relative importance of the factors just enumerated varies with different flours.

Summary

The temperature at which mixogram patterns of hard red spring wheat flours are made has an exceedingly important effect upon curve properties. Curve height and width were decreased by increasing the temperature. Dough development period was also markedly reduced by increasing the temperature except in the case of curves made with baking formula doughs at temperatures above 30°C. This appeared to be due chiefly to the low form of the mixograms. Varietal differences were more pronounced when the flours were mixed with the ingredients of the baking formula than when water alone was used.

The precise effect of temperature on curve height and width was not uniform for flours representing different varieties. Dough development stage, however, varied with temperature in essentially the same manner for all varieties. The precise effect of temperature variations on mixograms differed for flour-water and baking formula doughs.

Differences in protein content were less effective in changing mixogram properties than temperature variations. Curves resembling those of normal protein hard red spring wheat flours may be obtained from low protein flours by making the recordings at a lower mixing temperature.

Variations in curve pattern obtained by changing the flour absorption differed somewhat from those caused by a change in temperature. While an increase in absorption decreased curve height, and to a lesser extent curve width, the dough development stage was increased.

Acknowledgment

The authors wish to acknowledge the valuable assistance of Muriel Elledge in performing the statistical analyses.

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THE MEASUREMENT OF OVEN SPRING AS AN AID IN CONTROLLING FLOUR QUALITY

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(Received for publication May 20, 1944)

The measuring of oven spring by a device similar to that described by Whitcomb (1938) [1] has proved helpful when used in conjunction with the baking test during the routine testing of bread flour. Since oven spring is indicative of gas production and gas retention in a dough, it is logical that it should serve some purpose in controlling flour quality. The present report shows how this test has been used.

The device used by Whitcomb (1938) [1] for measuring oven spring was refined by making it possible to read the scale to the nearest millimeter. The device is shown in Figure 1. Among the flour samples tested with the device were short patents, stuffed straights, and first clear flours milled from Kansas wheat and patents and first clears milled from spring wheat. All baking tests were made using the Bread Baking Test for Wheat Flours as outlined in Cereal Laboratory Methods (4th ed., 1941). All doughs were proofed for the con-

[1] Whitcomb, W. O., 1938. Oven spring of dough as correlated with certain properties of bread. Cereal Chem. **15**: 206–216.

stant time of 55 min. The measuring of the oven spring was done as recommended by Whitcomb; however, readings were made to the nearest millimeter.

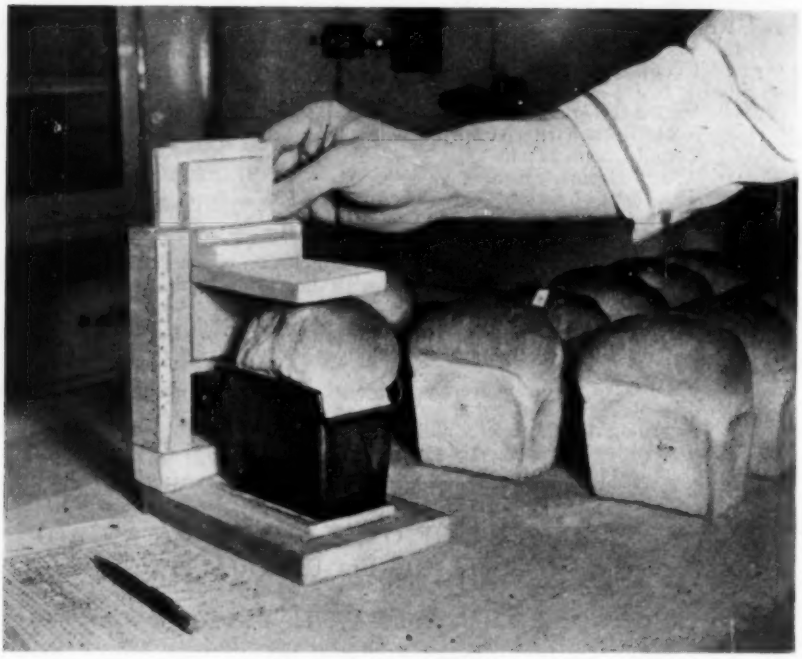

Fig. 1. Device for the measurement of oven spring.

The effect of increasing increments of malted wheat flour on a sample of wheat flour is shown in Table I. As malted wheat flour was added to the control flour, the height of the dough at the end of the proofing period increased. Also, the gassing power, as measured by

TABLE I

EFFECT OF INCREMENTS OF MALTED WHEAT FLOUR ON PROOFING HEIGHT AND GASSING POWER OF DOUGHS

Sample	Height of dough [1]	Gassing power [2]
	mm	*mm*
Control [3]	92	324
Control plus 0.2% malted wheat flour	100	413
Control plus 0.3% malted wheat flour	101	469
Control plus 0.4% malted wheat flour	103	502
Control plus 0.5% malted wheat flour	103	509

[1] Measured at end of proofing period.
[2] Gas pressure in mm Hg, as measured in the Sandstedt-Blish Pressuremeter during 5 hr fermentation at 30°C.
[3] The control was a non-diastated short patent flour milled from Kansas Wheat. The flour contained 12.0% protein and 0.40% ash, both expressed on a 13.5% moisture basis.

the Sandstedt-Blish Pressuremeter (Cereal Laboratory Methods, 4th ed., 1941), increased as the malted wheat flour was increased. This indicates that the height of the dough at the end of the proofing period depends to a great extent on the gassing power of the flour. Thus, the measurement of the height at the end of the proofing period with the oven spring device gives an indication of the gassing power of the flour. This measurement is not as sensitive as that given by the Sandstedt-Blish Pressuremeter; however, a severe deficiency or excess of gassing power is indicated by the height of the dough at the end of the proofing period.

In Table II, examples are given of two pairs of doughs, each pair having the same height at the end of the proofing period but differing

TABLE II

RELATION OF OVEN SPRING TO GAS RETENTION PROPERTIES OF DOUGH[1]

No.	Height after proofing	Height after baking	Oven spring	Loaf volume
	mm	*mm*	*mm*	*cc*
1	107	131	24	650
2	107	136	29	695
3	108	140	32	695
4	108	146	38	755

[1] All flours were short patents milled to the same specifications by different mills.

in their oven spring values. In each pair, the same height before going into the oven indicates to a great extent equal gassing power, but the dough having the greater oven spring must have a gluten better able to retain the gas during baking. In this way, the oven spring device is useful in indicating the relative gas retention properties of flour samples milled to the same specifications.

The data obtained from the device during routine test baking on the same type of flour serve at least two purposes:

First, the height of the dough at the end of the proofing period and just before going into the oven is usually indicative of the gassing power of the sample. Although the device has not been found sensitive enough to detect small differences in diastatic activity between samples, it is of service in pointing out flour samples having abnormally high or low gassing power. It should be realized, however, that measurements of height of dough are dependent on the elasticity of the gluten as well as the gassing power. Thus, an estimate of gassing power given by the oven spring device is not a simple measurement of gas evolution.

Second, the measurement of oven spring, which is the difference between the height of the dough before entering the oven and the height of the baked loaf, is of some value in judging the gas retention

properties of flour. As shown in Table II, the evaluation of gas retention properties by the oven-spring device is relative. When samples of flour, milled to the same specifications, are of about the same height before going into the oven, their oven spring values may be compared. With the aid of the oven-spring measuring device, some information in relation to the gas-producing and gas-retaining properties of flour may be obtained during the course of routine test baking.

The additional operations in using the device are few, and abnormal flour samples are quickly noted.

REPORT OF THE 1943–44 METHODS OF ANALYSIS SUBCOMMITTEE ON THIAMINE ASSAY [1]

JOHN S. ANDREWS, Chairman

General Mills, Inc., Research Department, Minneapolis, Minnesota

(Read at the annual meeting, May 1944)

The recently completed collaborative study of thiamine assay methods has differed somewhat from those carried out previously. In the past, collaborators have assayed samples by specific procedures and the results have been examined to determine how well the different laboratories agreed. While in many instances the agreement has been quite good, there have been a number of discrepancies which could not be accounted for. This has suggested the desirability of collaboratively studying the separate steps of the assay methods. How critical are these steps, and which might be expected to give rise to errors?

A canvass of 42 laboratories was made to obtain ideas about answering this question. The result was a long list of constructive suggestions covering nearly every phase of the thiochrome procedure. A few of the items were chosen for study. One relates to the efficiency of zeolite for removing interfering substances. Others consider factors relating to the conversion of thiamine to thiochrome; the effect of the amount of ferricyanide, the order of adding ferricyanide and alkali, the period of shaking, and the stability of the extracted thiochrome.

The Collaborative Committee's Methods

A complete description of the recommended procedures, together with a detailed discussion of the reasons for their selection, is best

[1] Paper No. 59, Journal Series, General Mills, Inc., Research Department.

afforded in the two communications addressed to the collaborators. For this reason they are reproduced below.

To the Members of the A.A.C.C. Collaborative Committee on the Thiochrome Method

Among the numerous questions raised by the collaborators about the thiochrome procedure are several relating to the quantitative aspects of the zeolite treatment of cereal extracts and the validity of the method employed for determining the value of the standard thiamine solutions used in calculating the vitamin content of the assay samples. Since considerable light can probably be thrown on both these problems by a series of recovery experiments, the following is being submitted for collaborative study. Two ampules of a standard thiamine solution containing 0.5 mg per ml (kindly supplied by Dr. Arnold of Winthrop Chemical Company) and samples of enriched flour and bread are being furnished for this work.

Experimental

One-gram samples of the enriched flour are weighed into each of six 250 ml flasks and suspended in 50 ml of 2% acetic acid containing, respectively, 0, 1, 2, 3, 4, and 5 μg of thiamine. To prepare these solutions, dilute 1 ml of the submitted standard to 500 ml with 2% acetic acid and in turn dilute 50 ml of this solution to 250 ml with the same solvent. Dilute 5, 10, 15, 20, and 25-ml aliquots of this second solution to 50 ml with 2% acetic acid and use for the extractions listed above.

Extract by heating on a steam bath for 15 min, taking precautions to prevent evaporation. Cool, add 5 ml of 1.5*N* sodium hydroxide and 5 ml of freshly prepared 6% takadiastase solution. Stopper the flasks and incubate at 37°C for 3 hr. Filter and pass 20 ml aliquots of the filtrates through zeolite in the manner you regularly employ. After washing and drying the zeolite, remove the thiamine with two 10-ml portions of hot 25% KCl in 2% acetic acid, collecting the first 15 ml. Mix thoroughly to insure homogeneity of this solution.

Transfer 5-ml aliquots to glass-stoppered cylinders or reaction vessels and add rapidly one drop (approximately 0.05 ml) of *0.5%* potassium ferricyanide solution, 3 ml of 15% sodium hydroxide, and 14 ml of isobutanol, quickly mixing after each addition. Shake vigorously for about 1 min, centrifuge, and discard the aqueous layer. Dry the isobutanol layer with sodium sulfate, transfer the *clear* solution to the curvette, and measure the fluorescence in the regular manner. Due to the rather wide range of thiamine represented by these extracts, the fluorescence of the extract containing no added thiamine should not be too high. A reading of below 30 galvanometer units should keep the fluorescence values of the other extracts on the galvanometer scale. After measuring the fluorescence values of the isobutanol solutions, determine the "blanks" in the usual manner. It will not be necessary to "run blanks" on all, since they should be identical. Two are sufficient unless discrepancies are noted.

Repeat the same experiment, using the sample of bread.

Notes

It will be greatly appreciated if each collaborator will follow a standard form in reporting his results. This will greatly facilitate subsequent analyses of the data. The attached sample sheet will illustrate the form desired. On it is recorded a typical set of data.

Under the column marked "Test," A and B represent duplicate experiments. Where time permits it is hoped that each collaborator will repeat the assays, preferably on different days, recording the results separately as indicated.

The "Fl.—orig." refers to the fluorescence values of the thiochrome solutions, and column "Fl.—Bl.," the "blank" values. Column III is simply the differences between the corresponding Fl.—orig. and Fl.—Bl. values, and column IV the differences between successive values in column III. These values (IV) represent the actual differences in fluorescence due to added 1-μg increments of thiamine, and the averages shown at the bottom of the column are the mean values used to calculate the assays in the manner shown below. Column V shows the amount of fluorescence due to the thiamine in the sample alone after deducting that due to the added vitamin. The first value, 31, is of course that determined directly (column III). The next value, 31.8, is obtained by deducting 7.2, the average increase due to 1 μg

A.A.C.C. COLLABORATIVE STUDY RECOVERY EXPERIMENT—ENRICHED FLOUR

Sample	Test	Fl.—orig.	Fl.—Bl.	III	IV	V
1-g sample	A	37	6	31		31
	B					
Plus 1 μg B_1	A	45	6	39	8	31.8
	B					
Plus 2 μg B_1	A	52	6	46	7	31.6
	B					
Plus 3 μg B_1	A	59	6	53	7	31.4
	B					
Plus 4 μg B_1	A	65	6	59	6	30.2
	B					
Plus 5 μg B_1	A	73	6	67	8	31
	B					
Averages	A				7.2	31.2
	B					
Final assay value	A					4.33 μg/g or
	B					1.97 mg/lb

of added B_1, from the corresponding value in column III. The next value, 31.6, is found by deducting 7.2×2 (2 μg of added B_1) from 46 in column III, etc.

The thiamine content of the sample is readily calculated from the averages in columns IV and V by dividing the latter by the former. Thus, $31.2 \div 7.2 = 4.33$ μg/g, or 1.97 mg/lb.

It should be pointed out that this study is proposed not as a method for assay but as an experiment to evaluate steps in the present assay procedure. No one would want to run so many levels of vitamin in a single analysis. In the present instance, the five levels of added vitamin are suggested for two reasons: (1) to increase the accuracy of the results and, (2) to study the effectiveness of the method over a fairly wide range of vitamin concentrations. The work can be simplified to some extent by decreasing the number of levels, and where the collaborator feels that this would expedite his work, it is suggested that the 2- and 4-μg levels of added B_1 be omitted. This, with the omission of duplicates, should enable all the collaborators to make some contribution. It is hoped, however, that the full program can be carried out and thereby insure the maximum value from the study.

In comparing the results from the flour and bread samples, it will be of considerable interest to note the relationship between the column IV averages of these two samples. If they are essentially identical, it can be assumed that thiamine is recorded similarly in both types of products. If they differ materially, it will be necessary to re-examine present procedures, at least as they pertain to the use of the assay standard. Your data will help materially to settle this question.

Anticipating that there may be some significant difference between the column IV values for flour and bread, one other short study is proposed. This merely comprises omission of the sample and carrying out the assays on the several levels of pure thiamine alone. How do the column IV values thus obtained compare with those for bread and flour? If you can include such an experiment in your study, it may add much to the significance of the results.

One more request. Just to bring your own methods into the picture, please include in your report an analysis of the two samples, using your own standard thiamine and your present technique.

Compare your standard with the one prepared from the solution submitted for this collaborative study.

In order to have time to assemble and analyze all the collaborators' data and prepare a report for the forthcoming A.A.C.C. convention, your results should be sent in as soon as possible. It will be greatly appreciated if this is done before March 15. Data received after April 1 will be too late for formal inclusion in the convention report.

JOHN S. ANDREWS, Chairman
Vitamin Assay Committee

To the Members of the A.A.C.C. Collaborative Committee on the Thiochrome Method

The replies received from the collaborators in response to the writer's request for comments on phases of the thiochrome method believed to need further study indicated a widespread uncertainty about the present oxidation procedures. Numerous questions have been raised about such factors as (a) optimum strength of the ferricyanide solution, (b) preferred order of adding alkali and ferricyanide, (c) optimum oxidation time and conditions, (d) stability of the thiochrome solution, etc. Because of these queries, one phase of the collaborative study has been designed to examine the above factors.

Two ampules of a standard thiamine solution containing 0.5 mg/ml (kindly supplied by Dr. Arnold of Winthrop Chemical Co.), together with a sample each of enriched flour and enriched bread, are being submitted for this collaborative work.

Experimental

In order to supply sufficient material for the oxidation studies, four 2-g samples of enriched flour (also enriched bread) are extracted with 50-ml portions of 2% acetic acid and the samples heated in a steam bath for 15 min, taking care to avoid evaporation losses. Five ml of 1.5*N* sodium hydroxide and 5 ml of freshly prepared 6% takadiastase solution are added to the cooled flask and the samples incubated for 3 hr at 37°C. After filtration, the four filtrates are combined and 20-ml aliquots are passed through 8 zeolite adsorption columns. The thiamine is removed from the zeolite with 25% potassium chloride in 2% acetic acid, 15 ml of solution being collected from each tube. These are then combined to give approximately 120 ml of solution for the oxidation studies.

Effect of Amount of Ferricyanide. Prepare a 4% solution of ferricyanide and dilute portions of this solution to give concentrations of 2%, 1%, and 0.5%, respectively. Place 5-ml aliquots of the combined zeolite-treated extracts in *glass-stoppered* cylinders or reaction vessels and oxidize, using one drop (approximately 0.05 ml) of these four different concentrations of ferricyanide solutions, adding the ferricyanide before the 3 ml of 15% sodium hydroxide. Add 14 ml of isobutanol in the regular manner, shake for one min, separate the two layers, dry the isobutanol layer with sodium sulfate, and determine the fluorescence. Report the fluorescence values for each of these oxidation experiments together with a blank value for the extract (both flour and bread samples).

Effect of Order of Adding Reagents. Following the above procedure, compare the fluorescence values obtained when the lowest (0.5%) and highest (4.0%) concentrations of ferricyanide are added *after* the addition of the sodium hydroxide. Also compare these results with those obtained when both ferricyanide and sodium hydroxide are mixed together before adding to the extracts. The solution for this latter experiment can be conveniently prepared by adding 5 drops of the ferricyanide solution to 15 ml of the sodium hydroxide and employing 3-ml aliquots of this mixture for the oxidation.

Effect of Time and Shaking. Using one drop of the 0.5% and the 4.0% ferricyanide solutions and adding the ferricyanide before the alkali, carry out one set of oxidations by merely inverting twice the oxidation mixture of extract, ferricyanide, alkali, and isobutanol. Allow to stand one min before separating the layers and determining fluorescence. Compare the results with those obtained above where one-min shaking was employed. Again using the lowest and highest concentrations of ferricyanide and the procedure in which ferricyanide is added before the alkali, compare the fluorescence values obtained when shaking is carried out ½ min, 2 min, and 4 min.

Stability of the Thiochrome Solution. Using your regular procedure for oxidizing thiamine solutions, oxidize three 5-ml aliquots of the flour and bread extracts, preparing the isobutanol solutions of thiochrome ready for fluorescence measurements. Read the fluorescence of the first at once, and allow the other two to stand on the desk for 30 and 60 min, respectively, before determining the fluorescence values.

Notes

Since the above experiments are designed to compare different types of oxidation treatments, your report needs only to record the fluorescence values of the isobutanol extracts. Don't attempt to calculate back to thiamine content of the sample for this work. In order to correlate the different collaborators' data, it will be desirable

to record the fluorescence of a standard thiamine solution. For this purpose, please use the submitted thiamine standard, diluting 1 ml (0.5 mg thiamine) to 100 ml with 2% acetic acid, and in turn diluting 10 ml of the resulting solution to 250 ml with 25% potassium chloride in 2% acetic acid. Five-ml aliquots of this potassium chloride solution should be oxidized directly and the fluorescence values of the thiochrome solution and the "blanks" reported.

Please include in your report thiamine values for the samples of enriched flour and enriched bread, following your regular assay procedure.

While the above program seems to present a rather large amount of work, it is hoped that each collaborator will complete it as far as possible. It would be best to carry out the complete study on the flour sample and, if time is then available, to do the same with the sample of enriched bread. Duplicate experiments would be very desirable but may be eliminated where the opportunity for doing this work is too limited.

It will be greatly appreciated if your results can be reported by the middle of March. Since it will take considerable time to organize the collaborators' data, reports received after the first of April will be difficult to include in the final report presented at the A.A.C.C. annual convention.

JOHN S. ANDREWS, Chairman
Vitamin Assay Committee

Results and Discussion

The data presented in Table I summarize the zeolite study carried out by examining the recovery of added thiamine. The first column shows the average fluorescence values due to the vitamin in the samples

TABLE I

THIAMINE RECOVERY EXPERIMENTS

Sample	Fluorescence			Assay
	Flour (blank deducted)	1 μg/g added B_1	Blank	
	Galv. units	*Galv. units*	*Galv. units*	*mg/lb*
Enriched flour	28.8	6.17	6.3	2.11 [1]
Enriched bread	22.2	6.22	6.7	1.63
Pure thiamine	—	6.10	6.0	—

[1] Calculated for enriched flour: 2.09 mg/lb.

of enriched flour and bread. The second column shows the corresponding values for 1-μg increments of added thiamine. It will be noted that these values for flour and bread are essentially identical. This indicates that, when passed through zeolite, both types of products have the same effect on the assay.

In order to determine the magnitude of this effect, the collaborators were requested to carry out recovery experiments using pure thiamine alone. The last value in the second column shows the result, 6.10 galvanometer units. This is slightly lower than those obtained from the flour and bread. Unfortunately, not all the collaborators reported results for the pure thiamine, and accordingly the value 6.10 is not strictly comparable with the others. If the comparison is confined only to those laboratories which carried out all three of the experiments,

the agreement is much closer. The values then become 6.10 for the flour, 6.08 for the bread, and 6.10 for the pure vitamin. The use of zeolite in the assay of thiamine does not appear to present any appreciable source of error.

The third column of the table shows the average blank values. It will be noted that they are approximately equivalent to 1 μg of thiamine. There was, however, considerable variation between the individual collaborators. One laboratory reported extremely low values, while another obtained values which were correspondingly high. The high blank did not appear to have any adverse effect, however, since the assay result was almost identical with the average. The fact that the blank values for the flour and bread are somewhat

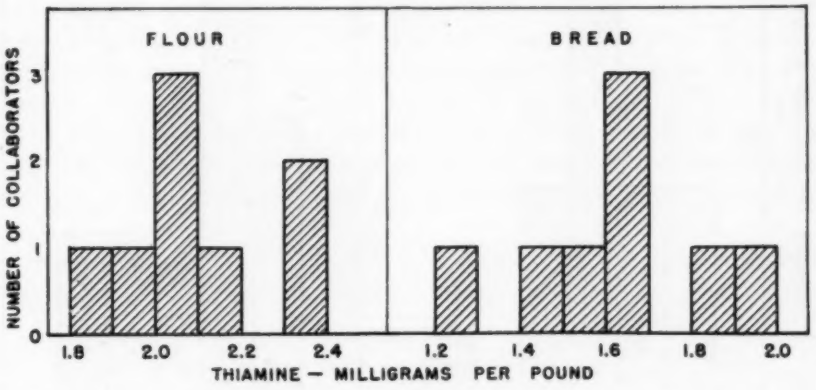

Fig. 1. Distribution of collaborators' thiamine assays using added thiamine for the standard.

higher than those for the pure thiamine indicates that the removal of interfering substances by the zeolite is not entirely complete. The greatest difference is seen in the sample of bread. However, in terms of the total fluorescence, this difference is quite small.

The last column shows the average assays for the two samples. The 2.11 mg/lb obtained for the flour very closely agrees with the calculated value, 2.09 (0.34 mg/lb native in the flour + 1.75 mg/lb of added thiamine). The value for bread is 23% lower.

The distribution of the individual collaborator's values is shown in Figure 1. Four, or 50%, of the values for the flour ranged between 2.0 and 2.2 mg/lb, *i.e.*, within ± 5%. Another collaborator was only slightly below this range (1.96 mg/lb). The lowest value was 1.82 mg/lb, and none of the data reveals the cause for this discrepancy. This collaborator obtained the same values for the added thiamine and the pure thiamine solution.

At least one of the two high values, ranging from 2.3 to 2.4 mg/lb,

can be attributed to an error in the standard. The submitted standard gave nearly 15% less fluorescence than this collaborator's U.S.P. thiamine. Use of the latter would have placed the assay within the ± 5% range. The other high value cannot be explained from the data. The collaborator did point out, however, that the actual work was carried out by an inexperienced technician.

The bread assays covered a somewhat greater range, 1.2 to 2.0 mg/lb. The values are based on the air-dried product, containing 9% to 10% moisture.

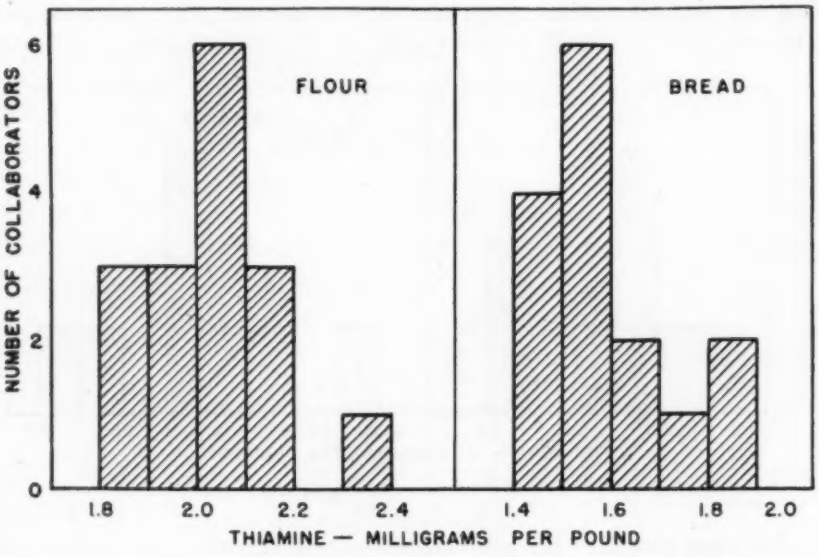

Fig. 2. Distribution of collaborators' thiamine assays using the regular thiochrome method.

Once again, 50% of the collaborators agreed within ± 5%. They did not all, however, represent the same laboratories which agreed on the flour. This makes it difficult to draw definite conclusions. For example, the inexperienced technician obtained an assay almost identical with the average. On the other hand, the lowest value for the bread was reported by a collaborator who obtained a flour assay within 2% of the calculated value. He attributes his low assay to an inadequate enzyme digestion period at too low a temperature. Digestion at 50°C for a longer period increased his value 11%, but still not enough to fall within the ± 5% range. The collaborator whose high assay of flour was attributed to discrepancies between standards was also high for the bread. The same correction would have brought his bread assay within the ± 5% range.

At the time the collaborators carried out the recovery experiments, they also assayed the samples by their regular procedures. In addition, eight other collaborators assayed the same samples. The results are summarized in Figure 2. The averages are very similar to those obtained from the recovery studies, 2.04 and 1.59 mg/lb for the flour and bread, respectively.

The highest value for the flour (2.38 mg/lb) came from another collaborator who reported discrepancies between the U.S.P. and submitted standards. In contrast to the previously mentioned discrepancy, the submitted standard was 10 to 15% *higher* than the U.S.P. preparation. This caused the assay by his regular procedure to be higher than that obtained from the recovery experiment. The fact that the latter was in excellent agreement with the calculated value suggests that the U.S.P. standard may have been in error, particularly since the same discrepancy was also obtained with the bread.

In both instances of the discrepancies between standards there is no proof about the correctness of one or the other. All other collaborators reported good agreement between their own and the submitted solutions of pure thiamine.

Another phase of the collaborative work involved a series of oxidation studies. Table II summarizes the effect of ferricyanide concen-

TABLE II

EFFECT OF CONCENTRATION OF FERRICYANIDE AND ORDER OF ADDING OXIDIZING REAGENTS

Order of addition	Fluorescence	
	0.5% ferricyanide	4.0% ferricyanide
	Galv. units	*Galv. units*
Ferricyanide first	53	45
Alkali first	57	52
Mixed	54	51

tration and the order of adding the ferricyanide and alkali. It will be noted that the highest fluorescence values were obtained when the alkali was added first and followed immediately by the ferricyanide. This is in agreement with the observations reported by the English investigators, Wang and Harris (1942).[2]

The lowest results were obtained when the order was reversed and the ferricyanide added first. The use of the mixed reagent gave intermediate results.

In some laboratories these differences arising from the different order of adding the reagents were insignificant. In others they were

[2] Wang, Y. L., and Harris, L. J., Further notes on estimation of vitamin B_1 by the thiochrome method. Chem. and Ind. **61**: 27–28, 1942.

quite marked. In only one instance did the addition of alkali first give a lower value, and this existed only at the lower concentration of ferricyanide. The 4% solution yielded the highest fluorescence values under the same conditions.

A preliminary study in the author's laboratory indicates that this effect of the order of adding the reagents is related to the concentration of ferricyanide. When the amount of oxidizing agent is kept low (one drop of 0.5% solution), either order yields practically the same result, provided the second addition is made immediately after the first. As the amount increases, however, the favorable effect of adding the alkali first is revealed. Under these conditions the fluorescence decreases only slightly in contrast to the much greater change which results when the ferricyanide is added first. This perhaps accounts for the observations made by Wang and Harris, since their procedure uses a considerable excess of ferricyanide.

TABLE III

EFFECT OF CONCENTRATION OF FERRICYANIDE AND TIME OF SHAKING

Shaking time	Fluorescence	
	0.5% ferricyanide	4.0% ferricyanide
	Galv. units	*Galv. units*
Inverting	38	29
½ min	54	47
2 min	55	47
4 min	55	47

When adding alkali first, care should be taken to follow with the ferricyanide promptly. Otherwise decomposition of the thiamine occurs, resulting in decreased fluorescence. This does not occur if the ferricyanide is added first. No change results, even when the addition of alkali is considerably delayed.

It appears desirable to avoid an excess of ferricyanide, regardless of the order of adding the reagents. In the present collaborative study, the amount employed was only about one-fourth that recommended in *Cereal Laboratory Methods* (4th ed., 1941). In only one instance did this appear to be inadequate, and this failure was attributed to excessive quantities of iron in the zeolite.

The effect of ferricyanide concentration and shaking time is summarized in Table III. The majority of the collaborators reported maximum fluorescence values after one-half min of shaking. Relatively few required longer periods. It is interesting to note that, on the average, extended periods of shaking were without significant effect.

The final phase of the collaborative study considered the stability of the isobutanol extracts of thiochrome. How promptly must the fluorescence of these solutions be determined? Six of the collaborators prepared three isobutanol extracts each of the flour and bread samples. One set was measured promptly after preparation and the others after standing on the laboratory bench for 30 and 60 min, respectively. All but one of the collaborators found no difference after 30 min, and in most instances good stability was observed during the entire 60-min period.

Summary

The thiochrome method as applied to the assay of thiamine in enriched flour and bread has been collaboratively studied. The use of zeolite apparently presents no appreciable source of error, since thiamine undergoes the base exchange similarly, both in pure solution and in cereal extracts. Assays of enriched flour yielded an average value (2.11 mg/lb) in very close agreement with that calculated from the assay of the unenriched flour and the added amount of pure thiamine (2.09 mg/lb). The average value for the bread made from the enriched flour was 23% below the flour value, suggesting that baking losses approximated this percentage figure. The possibility that some assay errors can be attributed to faulty thiamine standards is discussed.

The concentration of ferricyanide and the order of adding ferricyanide and alkali have some effect upon the assay. Particularly where a considerable excess of ferricyanide is employed, it is preferable to add the alkali first, followed immediately by the ferricyanide. One-half min shaking of the oxidation mixture resulted in the maximum fluorescence in most instances. Additional shaking has no significant effect. Isobutanol extracts of thiochrome appear to be sufficiently stable to present no problem in the fluorescence measurement.

Acknowledgments

The author wishes to express his appreciation to Miss Marilyn Cooney for her able assistance in preparing this collaborative program and to the following individuals who participated in the collaborative study: A. W. Alcock, Western Canada Flour Mills Co., Ltd., Winnipeg, Manitoba; A. Arnold, Winthrop Chemical Co., Rensselaer, N. Y.; R. T. Bohn, General Baking Co., New York City, N. Y.; H. M. Boyd, General Mills, Inc., Minneapolis, Minn.; E. E. Brown, Anheuser-Busch, Inc., St. Louis, Missouri; F. J. G. de Leeuw, Lucidol Corp., Buffalo, N. Y.; D. E. Downs, Hollywood Candy Co., Centralia, Ill.; M. W. Mead, National Grain Yeast Corp., Belleville, N. J.; R. B. Meckel, American Inst. of Baking, Chicago, Ill.; B. L. Oser, Food Research Laboratories, Inc., Long Island City, New York; W. L. Rainey, Commander-Larabee Milling Co., Minneapolis, Minn.; W. Reeder, Campbell-Taggart Research Corp., Kansas City, Mo.; J. Rosin, Merck and Company, Inc., Rahway, N. J.; L. Rosner, Laboratory of Vitamin Technology, Chicago, Ill.; L. T. Saletan, Schwarz Laboratories, Inc., New York City, N. Y.; P. H. Towers, The Higginsville Flour Mills, Higginsville, Mo.; R. W. Truesdail, Truesdail Laboratories, Inc., Los Angeles, Cal.

REPORT OF THE 1943-44 METHODS OF ANALYSIS SUBCOMMITTEE ON RIBOFLAVIN ASSAY[1]

JOHN S. ANDREWS, Chairman

General Mills, Inc., Research Department, Minneapolis, Minnesota

(Read at the Annual Meeting, May 1944)

The first of the present series of collaborative studies on riboflavin assay methods was carried out two years ago (Andrews, 1943). The results showed that, despite considerable variations between the individual assays, microbiological and fluorometric methods yielded comparable values.

The collaborative study was continued the following year (Andrews, 1943a) with major emphasis on a fluorometric procedure. Once again wide variations were revealed, but there was evidence that under properly controlled conditions the method was capable of producing satisfactory results.

One major source of error appeared to lie in the use of "Florisil." While some laboratories found this adsorbent fairly efficient, others experienced difficulties. This was attributed to a lack of uniformity in the Florisil, and the analyst was cautioned to check its efficiency before using it for assay purposes. It was recommended that this be done by examining the recovery of riboflavin added to cereal extracts.

The second collaborative study also considered the use of permanganate for destroying interfering pigments. It was suggested that permanganate treatment might be omitted in the assay of enriched flour. Bread, however, appeared to require this oxidation since the use of Florisil alone would not prevent excessively high results.

The present collaborative study abandoned further attempts to examine complete assay procedures. Instead, its purpose was confined to an evaluation of permanganate and Florisil treatments when *separately* applied to the purification of cereal extracts. Recovery experiments involving enriched flour and bread were carried out by 13 collaborators and three types of assays reported. One showed the effect of omitting all purification and illustrated the influence of interfering substances in patent flour and bread derived therefrom. The second showed the extent to which these interfering substances were removed by permanganate oxidation. The third presented a similar picture as reflected by adsorption on Florisil. In addition, six other collaborators presented assays carried out by microbiological methods. These results have been compared with those obtained fluorometrically.

[1] Paper No. 60, Journal Series, General Mills, Inc., Research Department.

The Collaborative Committee's Methods

A complete description of the recommended procedure, together with a detailed discussion of the reasons for its selection, is best afforded in the following communication addressed to the collaborators:

TO THE MEMBERS OF THE A.A.C.C. COLLABORATIVE COMMITTEE ON RIBOFLAVIN ASSAY METHODS

A survey of the opinions of the collaborators on the subject of riboflavin assays reveals that the major problems in the assay procedures concern the use of permanganate and Florisil. Doubt is expressed about the resistance of riboflavin to permanganate oxidation and the quantitative aspects of the Florisil adsorption. In addition, more rapid methods are desired, together with the avoidance of pyridine.

The best type of collaborative study appears to be one which will attempt to answer some of these problems and evaluate the role of the oxidation and adsorption steps. Accordingly, a series of experiments have been designed and tested in the writer's laboratory. It is believed that their application in the present collaborative study will go far to develop a short, simple assay method.

Two ampules of riboflavin (5.54 mg in each, generously supplied by Dr. Arnold of Winthrop Chemical Co.) together with samples of enriched flour and bread are submitted for this work.

Experimental

Direct Reading. Prepare the standard B_2 solution by dissolving contents of ampule (5.54 mg) in 554 g or ml of distilled water and dilute 10 ml of resulting solution to 100 ml (1 μg/ml).

Weigh into 125 ml Erlenmeyer flasks six 1.5-g samples of enriched flour. Suspend uniformly in 50 ml of 0.1*N* H_2SO_4. Add the following amounts of standard B_2 (1 μg/ml) solution: 0, 2, 4, 6, 8, and 10 ml, respectively.

Heat by autoclaving 15 min at 15 lb pressure, cool and adjust to pH = 4.3, using 10% NaOAc solution (requires approximately 6.3 ml). Transfer to 100 ml volumetric flask and make up to 100 ml with distilled water. Mix thoroughly and filter, discarding first approximate 10 ml filtrate. (Filtrate should be clear!) Measure fluorescence of aliquots (10 ml for Coleman and 15 ml for Pfaltz and Bauer fluorometers).

Prepare fresh, cold solution (in ice bath) of 5% hydrosulfite in 2% sodium bicarbonate and add 0.5 ml to each of above aliquots after the original fluorescence is determined. Mix quickly and determine fluorescence (blank).

Repeat the experiment, recording the duplicate values under B. (See *Notes.*)

Carry out the same study, using the sample of bread.

$KMnO_4$ Treatment. Take 25-ml aliquots of filtrates, either from the previous experiment or prepared anew, and add 0.2 ml 4% $KMnO_4$. Mix and stand 1 min. Add 0.2 ml 3% H_2O_2. Transfer 10- or 15-ml aliquot to curvette, depending on whether Coleman or Pfaltz and Bauer instrument is used, measure fluorescence and record on separate sheet, using same form as before (Fl_{orig} in column I). Add 0.5 ml hydrosulfite solution, mix, and again measure fluorescence (Fl_{b1} in column II).

Florisil Treatment. Take 20-ml aliquots of the filtrates and pass through the Florisil column (see *Report of Riboflavin Assay Committee*, Cereal Chemistry 20: 614, 1943, for use of this Florisil adsorption procedure). After washing and drying, elute the riboflavin with pyridine acetic acid solution, collecting 20 ml of eluate. Mix thoroughly.

Using 10- or 15-ml aliquots, measure fluorescence and record in column I. Add 0.5 ml hydrosulfite solution, mix, and measure Fl_{b1} (Record in column II).

Notes

It will be greatly appreciated if the collaborator will follow a standard form in reporting his results. This will greatly facilitate subsequent analyses of the data. The attached sample sheet will illustrate the form desired. On it is recorded a typical set of data.

EXAMPLE OF REPORT SHEET

(Please follow this form carefully. This will greatly facilitate analysis of the collaborators' data.)

Sample	Test	*Sample* (*i.e.*, enriched flour)						
		I Fl_{orig}	II Fl_{bl}	III $Fl_{corr\ bl}$	IV $Fl_{orig} - Fl_{corr\ bl}$	V Avg.	VI Fl_1 μg	VII Fl_{sample}
1. 1.5 g flour	A	14.4	1.0	1.1	13.3			
	B	14.2	1.0	1.1	13.1	13.2	—	13.2
2. Plus 2 ml B_2 std.	A	19.5	1.0	1.1	18.4			
	B	20.7	1.0	1.1	19.6	19.0	2.9	13.0
3. Plus 4 ml B_2 std.	A	25.1	1.0	1.1	24.0			
	B	26.2	1.1	1.2	25.0	24.5	2.8	12.5
4. Plus 6 ml B_2 std.	A	32.8	1.0	1.1	31.7			
	B	33.1	1.1	1.2	31.9	31.8	3.6	13.8
5. Plus 8 ml B_2 std.	A	37.1	1.0	1.1	36.0			
	B	39.5	1.0	1.1	38.4	37.2	2.7	13.2
6. Plus 10 ml B_2 std.	A	44.3	1.0	1.1	43.2			
	B	45.0	1.1	1.2	43.8	43.5	3.2	13.5
Average							3.0	13.2

Final assay value A 2.9 μg/g
B

Direct Reading. Under the column marked "Test," A and B represent duplicate experiments. The column Fl_{orig} refers to the fluorescence values of the riboflavin extracts, and Fl_{bl} in column II, the values for the "blanks." Column III contains the corrected "blank" values. These are obtained by multiplying the "blank" readings in column II by a correction factor (1.05 if a 10-ml aliquot is used and 1.03 if the aliquot is 15 ml). These corrected "blank" values are then subtracted from the original fluorescence values in column I and the differences are recorded in column IV. The corrected values in column III should be calculated to the nearest tenth decimal place only, since greater accuracy is not justified in the average fluorometric reading.

The average for the duplicate values in column IV is recorded in column V. In column VI, please record the fluorescence values for each 1-μg increment of added B_2. Thus, in the attached table (column VI) the first recorded value, 2.9, is obtained by subtracting the 13.2 (column V) for the sample alone from that containing 2 μg of added B_2 (19.0) and dividing by 2. The next value, 2.8, is the difference between the sample containing 2 μg of added B_2 (19.0) and that to which 4 μg of B_2 was added (24.5) divided by 2, etc.

In column VII, record the fluorescence values for the sample alone after deducting the fluorescence due to added riboflavin. These values are best calculated from the averages of duplicate experiments in column V and the final average (3.0) in column VI. The first value, 13.2, is, of course, the same as the corresponding value in column V, since it represents the extract with no added riboflavin. The next value, 13.0, is calculated by subtracting 6.0 (3.0, the average for 1 μg in column VI, multiplied by 2 μg) from 19.0 (column V); the next value, 12.5, by subtracting 12 (3.0 × 4) from 24.5, etc. These values are then averaged, yielding, in the above example, 13.2, identical with the value obtained from the actual reading of the extract containing no added riboflavin (column V). It may happen, however, that these values are not identical, in which case the average of column VII should give the more accurate assay.

To calculate the assay value of the sample, use the final averages of columns VI and VII. Since the fluorescence of the sample is 13.2 and the fluorescence of 1 μg of pure riboflavin under the same conditions is 3.0, the sample contains 13.2 ÷ 3.0 = 4.4 μg. Since the sample weighed 1.5 g, it contains 2.9 μg/g.

$KMnO_4$ Treatment. Two volume corrections are required here. The blank is corrected by multiplying by 1.05 or 1.03 as before and recording in column III. This corrected value is subtracted from Fl_{orig} (column I) and the difference recorded in

column IV. Then, to correct for the added $KMnO_4$ and H_2O_2, the values in column IV are multiplied by 1.016 and recorded in column IVa (not shown in example of report sheet). Actually, these corrections are very small relative to the accuracy of the usual fluorometer reading.

Data in columns V, VI, and VII are calculated the same as in previous experiment, using values in IVa rather than IV. Don't carry calculated values beyond tenth decimal place.

Florisil Treatment. Correct blank and record corrected values in column III. Subtract these values in column III from the corresponding values in column I and record the difference in column IV. Calculate values for V, VI, and VII as before.

While it is expected that some of the collaborators will be unable to carry out the complete program, it is hoped that all who can will do so. Carefully done work from many laboratories will go far in guiding the final selection of an accurate assay procedure. If, however, you feel that you can't complete the study outlined above, do as much as you are able.

Since a major purpose of the study is to determine the value of the $KMnO_4$ and/or Florisil treatments, it will be more desirable to carry out part of each of the three experiments rather than to do one of them completely, if you find that some abbreviation of the program is desirable. It is suggested that, in this event, you omit the 2-, 6-, and 10-μg increments of added riboflavin and even omit the duplicate determinations if necessary.

Please assay the flour and bread samples by the method you are now using and report with the other results.

In order to allow time to assemble and analyze all the collaborators' data and prepare a report for the forthcoming A. A. C. C. convention, your results should be sent in as soon as possible. It will be greatly appreciated if this is done before March 15. Data received after April 1 will be too late for formal inclusion in the convention report.

JOHN S. ANDREWS, Chairman
Vitamin Assay Committee

A summary of the data obtained from recovery experiments on enriched flour is presented in Table I. The figures shown are the averages obtained when several increments of riboflavin were added to the cereal sample. The first column compares the fluorescence values

TABLE I

RECOVERY EXPERIMENTS—RIBOFLAVIN ADDED TO ENRICHED FLOUR

(Average values of 13 collaborators)

Treatment of extract	Fluorescence due to flour (blank deducted)	Fluorescence due to 1 μg/g of added riboflavin	Assay result
	Galv. units	Galv. units	μg/g
None	9.13	2.18	2.78
Permanganate	9.18	2.21	2.75
Florisil	7.23	1.57	2.98

Calculated riboflavin content of sample: 2.76 μg/g

due to the sample alone. These were obtained by deducting the hydrosulfite blanks from the total fluorescence of the variously treated extracts. It will be noted that permanganate oxidation has no significant effect, since the values before and after this treatment are essentially the same. On the other hand, the value resulting from treatment with Florisil is significantly lower, suggesting that this adsorbent removes fluorescent material. Such a conclusion, however,

is hardly valid in view of a factor which prevents direct comparison with the preceding values. The fluorescence was determined on acidified pyridine extracts and under such conditions the fluorescence, at least of riboflavin, is depressed considerably.

The second column shows the extent to which fluorescence was increased by 1-μg increments of added riboflavin. Once again it will be noted that treatment with permanganate had no significant effect. This observation leads to two conclusions. First, that permanganate had little, if any, value for the purification of extracts of patent flour, and second, that this oxidation did not result in destruction of riboflavin.

The value obtained after adsorption on Florisil is again low and is due not to any losses of riboflavin, but rather to the decreased fluorescence in the pyridine solution. Preliminary studies carried out in the writer's laboratory on pure riboflavin solutions indicated that this decrease amounted to about 25%. This would account for most of the differences noted in the tabulated values.

The last column shows the average assay results obtained from the recovery experiment. There is practically no difference between those obtained with and without permanganate. In both instances the assays are in excellent agreement with the calculated value, 2.76 μg/g. The result obtained by treatment with Florisil is too high.

Figure 1 shows how the individual values were distributed. It is obvious that greatest uniformity resulted when no purification treatment was applied. With the exception of two collaborators, the reported assays were between 2.6 and 2.9 μg/g. Introduction of permanganate oxidation, while not significantly changing the average, appeared to present difficulties resulting in somewhat poorer agreement between laboratories. A more thorough examination of this treatment appears desirable.

The results obtained by using Florisil were the most erratic. The values ranged between 2.2 and 3.6 μg/g. It is unfortunate that a uniform supply of the adsorbent was not made available to all the collaborators. Had this been done, it might have been possible to determine the source of these discrepancies. At present it is not known whether the difficulty was due to the Florisil or to faulty technique in its application. There is increasing evidence that the former was at least partly at fault.

Perhaps the most significant result of these recovery experiments with enriched flour is the suggestion that relatively simple assay methods can be employed. Extraction with dilute acid, filtration, and measurement of the fluorescence appear to be all that is required. "Blanks" can readily be determined with hydrosulfite and reference

standards established with pure riboflavin. Whether the riboflavin can be used directly or should be added to the flour is not definitely known. However, this can be readily determined by a few comparison experiments.

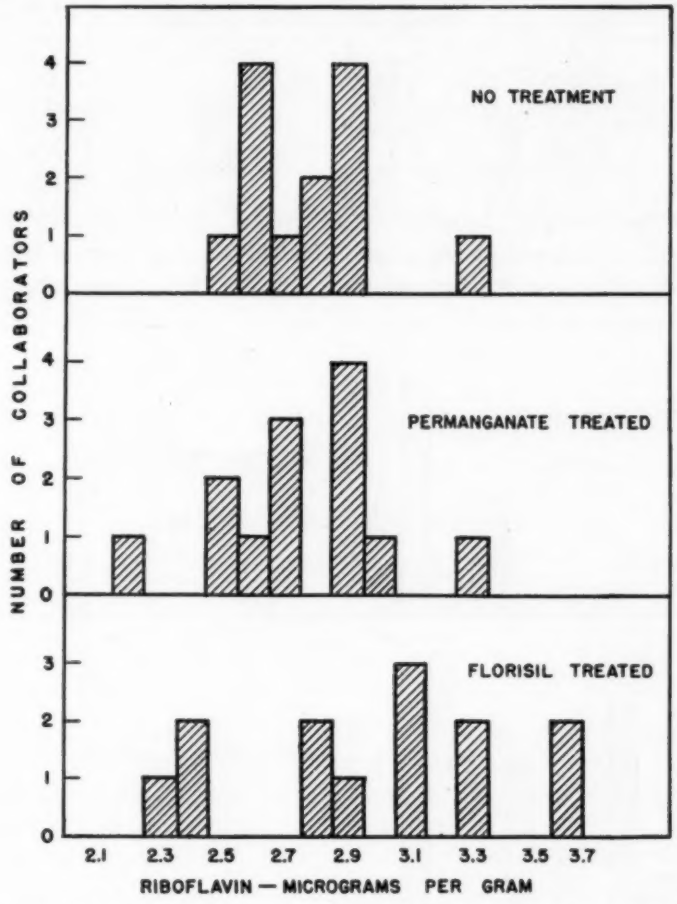

Fig. 1. Distribution of collaborators' riboflavin assays of enriched flour in which the flour extracts were subjected to different treatments.

A summary of the collaborative study on enriched bread is shown in Table II. These data demonstrate that bread presented a more complex problem than flour. The fluorescence values due to the bread alone indicate that permanganate treatment was beneficial. The decreased fluorescence resulting from this oxidation can be attributed to the removal of interfering impurities. This result is reflected in the values obtained for the 1-μg increments of added riboflavin. The treatment resulted in an increase over that obtained directly on the

TABLE II

RECOVERY EXPERIMENTS—RIBOFLAVIN ADDED TO ENRICHED BREAD

(Average values of 13 collaborators)

Treatment of extract	Fluorescence due to bread (blank deducted)	Fluorescence due to 1 μg/g of added riboflavin	Assay result
	Galv. units	Galv. units	μg/g
None	12.49	2.07	4.08
Permanganate	10.87	2.17	3.42
Florisil	9.34	1.53	4.08

Calculated riboflavin content of sample: 3.16 μg/g

extract and brought the value into excellent agreement with the 2.18 obtained on the sample of flour. The final assay results were also affected. The use of permanganate lowered the average assay more

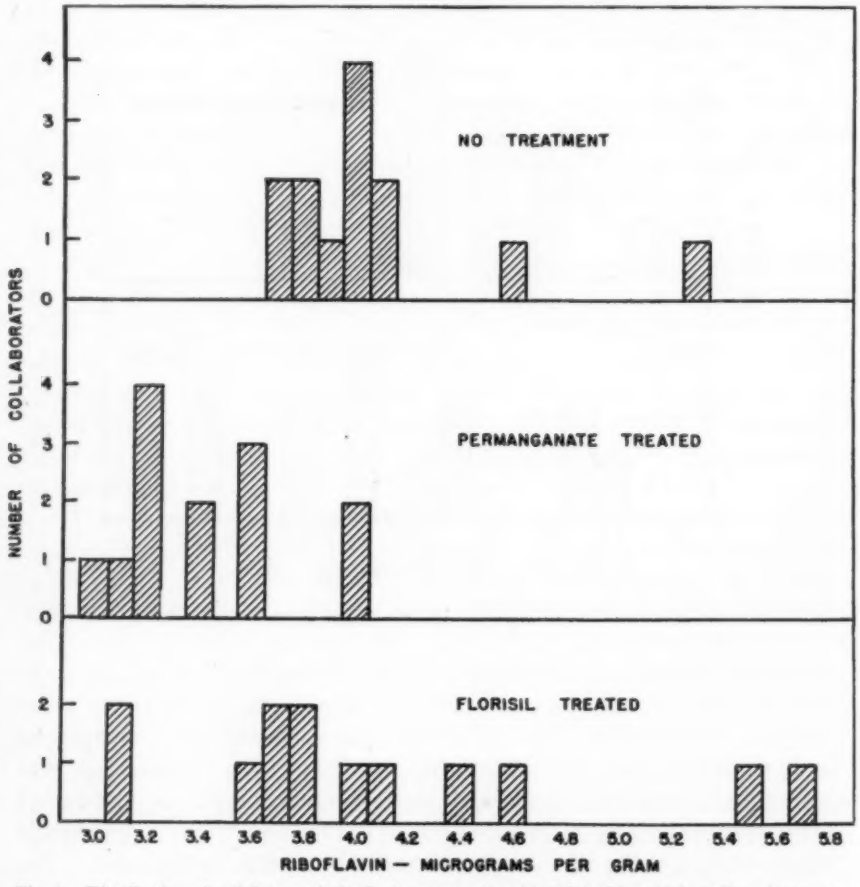

Fig. 2. Distribution of collaborators' riboflavin assays of enriched bread in which the bread extracts were subjected to different treatments.

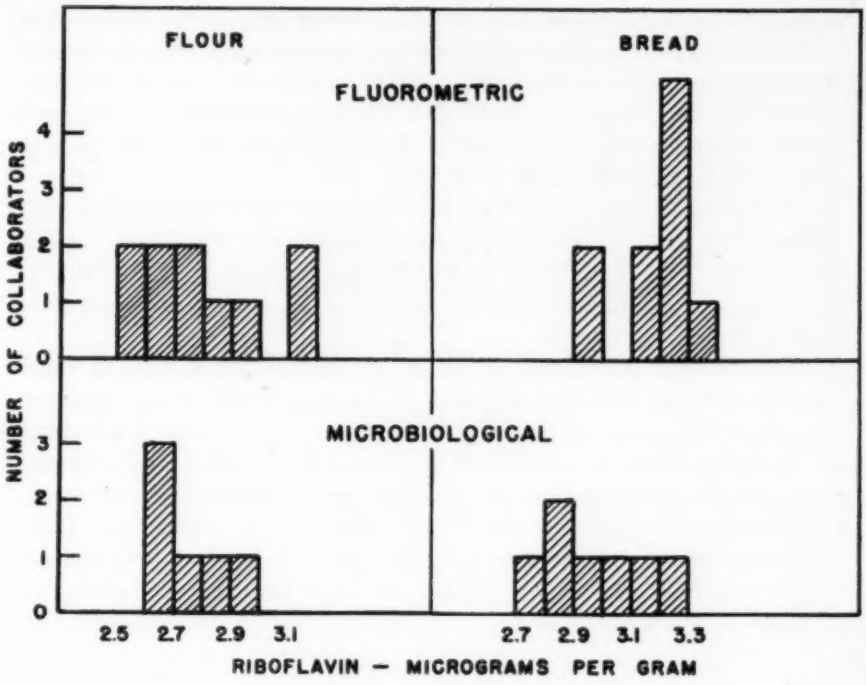

Fig. 3. Distribution of collaborators' riboflavin assays of flour and bread as determined by the fluorometric and microbiological procedures. The mean values are as follows:

	Riboflavin content	
	Fluorometric	Microbiological
	μg/g	μg/g
Flour	2.78	2.74
Bread	3.14	2.96

than 16% and brought it into much better agreement with the calculated value, 3.16 μg/g. This calculated value was based on the value for flour plus 0.42 μg/g added by the yeast. It is expressed on an air-dry basis (9.5% moisture).

As in the instance of the flour, the use of Florisil was of questionable value. The assay obtained by treatment with this adsorbent was excessively high and the same as that obtained directly on the extract. It should be remembered, however, that this experiment differed from the complete assay procedure. Florisil was used alone and not in conjunction with the permanganate. It can only be concluded that Florisil *when used alone* is not particularly effective.

The distribution of the individual collaborator's values is indicated in Figure 2. The results obtained by omitting purification treatment are quite uniform if the high values obtained by two collaborators are not considered. They range from 3.7 to 4.1 μg/g, or practically within ± 5%. Treatment with permanganate significantly lowered the as-

says, but contributed nothing to better uniformity. About half the collaborators were in close agreement with the calculated value, but the remainder tended to be too high. The results from the treatment with Florisil are too erratic to carry any significant meaning. Only two collaborators closely approached the calculated value. The others were widely scattered on the high side.

In addition to the recovery experiments, 16 collaborators reported fluorometric and microbiological assays carried out by their regular procedures. The results are summarized in Figure 3. They are, in general, quite satisfactory, as can be seen by the averages for the several assays.

For the flour, the fluorometric method yielded 2.78 μg/g. The average obtained microbiologically was 2.74 μg/g. These values compare very favorably with the calculated 2.76 μg/g. The fluorometric value for the bread, 3.14, was equally in good agreement with that calculated from the bread ingredients, 3.16 μg/g. The value obtained microbiologically was about 6% lower (2.96 μg/g). The distribution of the individual values is shown graphically and represents a decided improvement over the results obtained from previous collaborative studies.

Summary

The effect of permanganate and Florisil as employed separately in the fluorometric analysis of enriched flour and bread has been collaboratively studied. In the instance of enriched flour it appears that neither of these two purification treatments offers any value. Direct reading of flour extracts gave just as satisfactory a result as when either permanganate or Florisil were employed. Direct assay of the collaborative sample of enriched flour yielded a value of 2.78 μg/g, in excellent agreement with the calculated value, 2.76. When the extracts were absorbed on Florisil and eluted on pyridine a somewhat higher value was obtained, indicating the inadequacies of the Florisil.

In the instance of enriched bread the use of permanganate aids considerably in removing interfering impurities. This treatment was not entirely effective, however, since the average value obtained was 3.42 μg/g, as compared to the calculated value, 3.16. Treatment of the extract with Florisil had no effect on the final results, the same value being obtained as when the extracts were read directly.

Values reported by the collaborators employing their regular procedures were in general quite satisfactory. Both fluorometric and microbiological methods were represented and, particularly in the instance of flour, yielded values which closely agreed with the calculated values. Fluorometric assays of enriched bread were somewhat higher

than those obtained microbiologically and were in closer agreement with the value calculated from the bread ingredients.

Acknowledgments

The author wishes to express his appreciation to Miss Jane Spence for her able assistance in preparing this collaborative program and to the following individuals who participated in the collaborative study: H. Boeddeker, General Mills, Inc., Minneapolis, Minn.; H. M. Boyd, General Mills, Inc., Minneapolis, Minn.; W. Brew, Ralston Purina Co., Inc., St. Louis, Mo.; R. T. Conner, General Foods Corp., Hoboken, N. J.; C. N. Frey, Fleischmann Laboratories, New York City, N. Y.; D. Glick, Russell-Miller Milling Co., Minneapolis, Minn.; C. G. Harrel, Pillsbury Flour Mills Co., Minneapolis, Minn.; L. C. Norris, Cornell University, Ithaca, N. Y.; L. W. Haas, The W. E. Long Company, Chicago, Ill.; S. H. Rubin, Hoffmann-LaRoche, Inc., Nutley, N. J.; R. A. Stewart, Quaker Oats Company, Chicago, Ill.; H. B. Wigman, Mellon Institute, Pittsburgh, Penn.; V. O. Wodicka, Chicago Quartermaster Depot, Chicago, Ill.

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REPORT OF THE 1943–44 COMMITTEE ON TESTING BISCUIT AND CRACKER FLOURS

FRANK R. SCHWAIN, Chairman

Kroger Grocery and Baking Co., Columbus, Ohio

(Read at the Annual Meeting, May 1944)

There is a definite need for a reliable, sensitive cooky-baking test to supplement analytical data, particularly the viscosity test, in evaluating cooky flours for specific baking applications. For the last three years the committee has been developing a test-bake formula for cooky flours which would differentiate such flours satisfactorily. As an outgrowth of these studies it became apparent that spread potentialties may vary with the white wheat varieties from different localities (Hanson, 1943). There were also indications that granulation in itself can change the spreading properties of a given flour (Loving, 1942; Hanson, 1943a). If this phenomenon could be manifested in practice, granulation might well be added to cooky flour specifications.

This year, then, the committee decided to attempt to translate the predictions of its cooky test into actual performances in bakeries. In addition it planned to determine if possible the practical differences in performance of flours from different areas or of various granulations. In order to accomplish this most efficiently, the work was so allocated

as to place specific tasks with those individuals whose everyday duties were closely allied to similar assignments. Flour preparations and complete analyses were in the main referred to mill-chemist members. Cooky baking tests and subsequent flour classifications were assigned to three different testing laboratories. Shop performances were allotted to those committeemen actively connected with four different cooky bakeries. It was felt that the findings then should command the interest of a larger portion of our membership.

Because of the acute shortage of Michigan wheat this year, the original plan to utilize most of the white wheat varieties, which had been considered by previous committees, had to be abandoned, and Pacific Coast wheat flours of three different granulations were studied. Analytical data for the flours, which were all unbleached and of 100% extraction, are given in Table I. Flours A and B came from the

TABLE I

ANALYSES OF TEST FLOURS

Flour[1]	Ash (15% mb)	Protein (15% mb)	Apparent viscosity			Maltose[2] (Approximate)
			No-time	1 hr digestion	2 g protein	
	%	%	°*MacM*	°*MacM*	°*MacM*	
A	0.40	7.65	35	48	66	221
B	0.37	7.85	39	56	74	209
C	0.41	7.70	36	53	74	203

[1] Flours A and B from same wheat mix differing only in grinding and bolting operations in milling.
[2] Maltose reported from Pressuremeter results (Conversion factor 1.7).

same wheat mix, but by means of changes in grinding and bolting operations, a difference in granulation was secured. Flour C was supplied by another mill. The flours are quite similar in composition. With granulation given the prime consideration, however, this factor was determined by two methods. The first method was that followed by the last two committees and consisted of measuring the "throughs" of a Ro-Tap testing sieve shaker fitted with a 250-mesh wire screen. The second method was another Ro-Tap test in which the main fractions were separated by a Ro-Tap clothed with five silks of different mesh. The results are given in Table II.

There is less difference in granulation in the test flours this year than formerly. Previous committees have worked with Michigan flours which differed as much as 11% in the "throughs" of a 250-mesh wire. Coast wheat flours have usually been the coarsest of all. This time the simplified method showed a differential of only about 6% between the coarse-ground and fine-ground products. The relatively narrow spread in granulation between flours A and B was attributed

TABLE II

RO-TAP GRANULATION STUDIES OF TEST FLOURS

Flour	Wire screen study through 250-mesh[1]	Silk sieve classification					
		Over 11 XX	Over 12 XX	Over 13 XX	Over 14 XX	Over 15 XX	Through 15 XX[2]
	%	%	%	%	%	%	%
A	86	3.9	22.1	32.4	27.2	11.8	2.6
B	91	3.1	20.0	27.2	25.1	18.3	6.3
C	85	3.6	19.0	23.8	28.5	21.4	3.7

[1] 250-mesh = 25 XX = 0.0024" opening.
[2] 15 XX silk = 170-mesh = 0.0037" opening.

by the mill to the quality of the Pacific Coast crop which required less tempering water to secure the flour desired, with the result that the middlings pulverized more readily than expected. The more comprehensive study employing silks would place flour C intermediate in granulation between A and B.

Again, the same laboratory procedure and test-bake formula described by Hanson (1943) were followed. The W/T or spread factors secured by the three collaborating laboratories are shown in Table III.

TABLE III

LABORATORY SPREAD FACTORS (W/T) OF FLOUR SERIES

Flour	Collaborator I	II	III
A	8.5	8.2	6.8
B	8.3	8.1	6.8
C	9.1	9.1	7.3

Without exception, flour C exhibited the best spread, with both A and B close behind. Although the coarser flour (A) appeared slightly better than the finer-ground, the advantage was not nearly so marked as in last year's Michigan flours, for example, when there was a greater difference in granulation.

Regular shop tests were also made in four different bakeries, utilizing formulas in current use and under normal operating conditions. These tests comprise Band and Reel oven performances. Three distinct types of products were made by the bakeries—wire cut cookies, rotary goods, and cutting pieces. Observations on spreading properties were determined 30 min out of the ovens. Table IV shows the results secured.

In the case of wire cut cookies, flour C performed the best in all four shops. Neither A nor B worked as well, both rating about the

TABLE IV

SHOP SPREAD FACTORS (W/T) OF FLOUR SERIES

Flour	Collaborator					
	IV (Band oven)	V (Reel oven)		VI (Reel oven)	VII (Reel oven)	
WIRE CUT COOKIES						
A	3.2	7.8[1]	5.5[1]	7.0	3.6	4.1
B	3.2	8.0[1]	5.7[1]	7.2	3.8	4.0
C	3.5	8.2	5.9	7.9	4.5	4.4
ROTARY GOODS						
					(Band oven)	
A	7.5	5.9		8.4[2]	7.8	10.1
B	7.5	5.8		7.6[2]	8.0	10.2
C	7.4	6.1		7.4[2]	8.3	10.2
CUTTING PIECES						
					(Band oven)	
A	6.9	10.4			8.2[2]	
B	6.5	10.4			7.8[2]	
C	6.7	10.8			8.3[2]	

[1] This collaborator added 2.5 lb sugar per 100 lb flour to flours A and B—wire cut only.
[2] Formula used "lean"; all others "medium."
Note—Where collaborator tried several kinds of wire cut or rotary cookies the results are listed side by side.

same. This is in fairly good agreement with the laboratory findings. Collaborator V deliberately incorporated 2.5 lb additional sugar per 100 lb of flour for both A and B wire cut doughs, but did not secure as good a spread in either case as that shown by the flour C dough without additional sugar.

Rotary goods, however, appeared less discriminative as to flour type.

TABLE V

CHECKING STUDIES OF FLOUR SERIES

Flour	Number of checked cookies in 100 after 24 hr			
	Collaborator V	Collaborator VII		
		Lot 1	Lot 2	Lot 3
A	17	33	24	22
B	29	26	19	67
C	12	7	17	6

Cutting pieces, on the other hand, favored flour C in two out of three shops with an indication of poorer performance on the part of the finer-ground flour (B). This compares favorably with laboratory predictions.

Checking or evidence of cracking was also considered. Each bakery was asked to determine the number of cookies per hundred, which showed signs of checking 24 hr out of the oven. Two shops experienced no checking. The observations made by the other two plants on their more brittle types of goods are summarized in Table V.

In these frangible pieces, far less checking was observed from flour C than from either A or B. This bears reinvestigation. The evidence presented is too limited to draw any accurate conclusions, though milling practices may have some bearing.

Conclusions and Recommendations

It would appear, from the data presented thus far, that the current laboratory test formula devised by earlier committees has application where flours intended for wire cut or sheeter cooky production are concerned. However, for rotary goods, the test appears to have less value and the results should be viewed with some hesitancy in evaluating flours for this purpose. This is logical in view of the larger amount of water usually required for sheeter and wire cut goods which compares more favorably with the water content of the laboratory test formula.

There are also indications with wire cut goods and sheeter cookies that milling technique may be a factor in explaining differences in performance in spite of similar wheat types and analyses. The phenomenon of checking should not be overlooked from the same standpoint.

Coarse-ground flour may exhibit better spreading characteristics than the same flour finely ground, if there is a more significant difference in granulation than that experienced here. Further experimentation should answer this point.

The committee suggests that the present laboratory test formula appears to be adaptable for differentiating flours intended for wire cut and sheeter production. For rotary purposes, a new formula may have to be developed which requires far less water; for example, 6 or 7%. Perhaps the answer lies in some sort of laboratory-size die for cutting out the cookies under pressure. This would more nearly approximate practical conditions.

The committee recommends additional studies of the importance of granulation in relation to the quality of cooky flours. The two

methods of measuring granulation employed here should be further investigated to determine which has the greater practical significance.

Correlating laboratory results with shop tests is not only desirable but necessary.

Flours from the white wheats of such areas as New York, Michigan, and the Pacific Coast certainly bear further consideration to determine whether the differences noted in previous laboratory tests are manifested from crop to crop and are actually demonstrable in commercial production.

Acknowledgments

The chairman wishes to express his appreciation of the excellent cooperation accorded him by the committee which included Miss Pearl Brown, W. H. Hanson, T. E. Hollingshead, Jan Micka, H. M. Simmons, O. P. Skaer, and L. S. Thomson. Acknowledgment is also made of the work done by P. W. Hodler. Appreciation is due Henkel Flour Mills, Centennial Flouring Mills, Perfection Biscuit Company, Kroger Grocery and Baking Company (Columbus Unit), and the Sawyer and Strietmann Divisions of the United Biscuit Company of America for their individual contributions.

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REPORT OF THE 1943–44 METHODS OF ANALYSIS SUBCOMMITTEE ON THE DETERMINATION OF IRON IN CEREAL PRODUCTS

M. Howe, Chairman

Russell-Miller Milling Company, Research Laboratory, Minneapolis, Minnesota

(Read at the Annual Meeting, May 1944)

The lack of agreement in the determination of iron has long been apparent from previous collaborative studies. This could be due, among other things, to the preparation of the ash for the determination of iron or to the contamination of iron from dishes and tongs. There is the possibility of nonuniformity of mixing of the enriched flour, but in this report the variation on the unenriched flour was as great as on the enriched. Iron can easily become a source of contamination, but this would not account for the very low results so often encountered. Such results could be explained by the presence of pyrophosphate in the ash, since the presence of pyrophosphate affects the color develop-

ment and must be converted to orthophosphate. Iron contamination from dishes used for ashing can be a serious problem; therefore, platinum or glass dishes are the most suitable. The dishes should be cleaned by dipping them successively in two or three boiling hydrochloric acid solutions, and cleaning should be continued until a blank determination carried out on the dish alone gives a negligible coloration. Large blanks on platinum dishes have been found if they were previously used for fat determinations, and just one boiling with hydrochloric acid will not remove all the contaminating iron present. Glass or platinum tongs should be used to handle the crucibles.

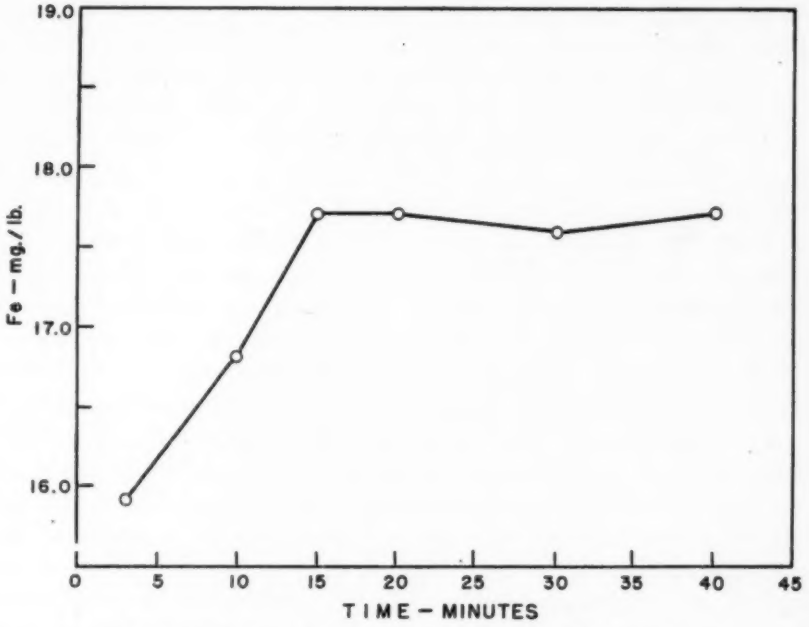

Fig. 1. Influence of time of digestion of ash from whole wheat flour with boiling hydrochloric acid solution (1 : 1) on the apparent iron content of the flour.

Preparatory to sending out the samples for collaborative work, a study was made of the heating period necessary to convert the pyrophosphate to the orthophosphate in the ash of whole wheat flour. The result of this study is shown in Figure 1. The time of heating was varied from 3 min to 40 min. The minimum time of heating required to secure the maximum iron value was found to be 15 min; however, 30 min was specified in the method sent to the collaborators to insure a reasonable safety factor.

Figure 2 shows a spectral transmission curve for the α,α'-dipyridyl complex. This was obtained with a Beckman Spectrophotometer.

The maximum absorption band was found to be at 520 mμ. The collaborators, therefore, were instructed to use an appropriate filter for this range.

In a continuation of the work of the 1942–43 Subcommittee on Methods of Analysis of Iron (Howe, 1943), the Committee undertook

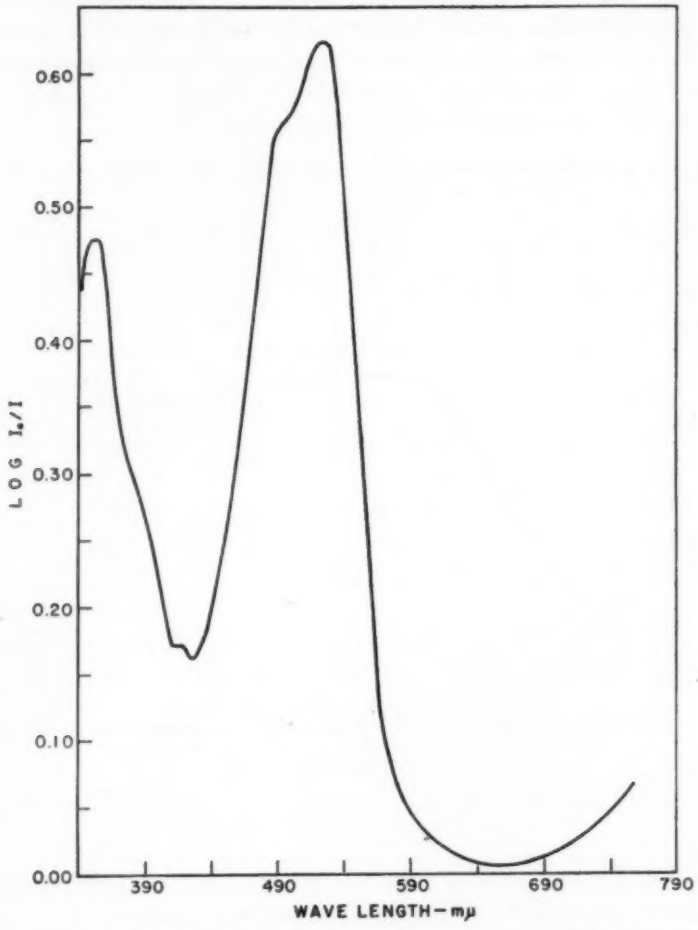

Fig. 2. Spectral distribution curve for the α, α′-dipyridyl complex (prepared from a standard iron solution containing 10 μg Fe per ml).

a study of the preparation of cereal products by various methods including hydrochloric acid treatment of the ash, sodium carbonate fusion, and wet digestion preparatory to the determination of iron by the α,α′-dipyridyl method. This study was made to ascertain the validity of dry ashing of cereal products and to compare the numerous methods now employed in various laboratories as routine determina-

tions with the α,α'-dipyridyl method. The samples sent to the collaborators comprised (1) an unenriched flour; (2) the same flour enriched with 10 mg of iron as ferrum reductum; (3) a bread made from the enriched flour; (4) a whole wheat flour; and (5) a bread made from the whole wheat flour.

The samples were prepared with a great deal of care, and the flour enriched with ferrum reductum was thoroughly mixed; in spite of this fact, several collaborators stated that the sample was not uniform. The whole wheat flour was passed over a magnet to remove any small pieces of metal which might have been present from the milling rolls, as several laboratories have reported finding such small pieces of metal in previous samples of whole wheat flour. The bread was baked, using an average formula, air dried, and ground in a mortar.

The three methods of preparation were as follows: (1) The dry ashing was accomplished by ashing the products at 550–600°C overnight, taking the ash up in 2 ml of HCl, heating for 30 min, filtering, and making up to 100 ml; (2) for the sodium carbonate fusion, the samples were ashed in the usual manner, then mixed with 0.5 g of purified sodium carbonate, and fused. The melt was taken up in 5 ml of 1 : 1 HCl, heated for 30 min, filtered, and made up to volume. (3) The wet ashing procedure was that of Jackson (1938). This method is time-consuming and was not employed with the intention of using it as a routine method, but because the Committee wanted to compare the results of dry versus wet ashing. In our laboratory, a loss of iron upon dry ashing of cereal products has never been observed, and this fact has been substantiated by other collaborators (Andrews and Felt, 1941). The collaborators were also requested to send in the results as determined by the method used routinely in their own laboratories; these included the use of ortho-phenanthroline, potassium thiocyanate, and numerous variations of the α,α'-dipyridyl method. The summarized results are given in Tables I–VI.

The following conclusions are evident:

Wet ashing did not consistently give higher results than dry ashing.

There was no particular advantage in the sodium carbonate fusion.

Individual laboratory methods did not give any closer agreement than the method suggested by the Committee.

The use of the α,α'-dipyridyl method with dry ashing is as satisfactory a method as is now available for the determination of iron in cereal products. More practice and improvement in technique should bring better agreement between laboratories.

TABLE I

IRON CONTENT OF UNENRICHED FLOUR

Iron content as Fe in mg/lb ("as is" moisture basis—12.6%)

Collaborator number	Hydrochloric acid treatment	Sodium carbonate fusion	Wet digestion	Individual laboratory method
1	4.0	4.3	4.0	—
2	3.6	3.6	—	—
3	2.8	3.1	—	4.1
4	—	—	—	5.0
6	3.9	3.9	5.7	3.9
7	4.0	4.1	4.2	—
8	4.3	4.4	4.4	4.3
10	3.6	3.8	3.6	4.2
11	4.0	4.0	—	4.1
12	3.9	3.5	—	—
13	3.3	4.2	—	—
14	4.4	5.1	—	—
15	3.8	3.5	3.8	—
16	—	—	—	4.4
17	—	—	—	3.8
Maximum	4.4	5.1	5.7	5.0
Minimum	2.8	3.1	3.6	3.8
Mean	3.8	3.9	4.3	4.2
Standard deviation	0.434	0.523	0.749	0.369

TABLE II

IRON CONTENT OF FLOUR ENRICHED WITH 10 MG/LB OF IRON AS FERRUM REDUCTUM

Iron content as Fe in mg/lb ("as is" moisture basis—12.6%)

Collaborator number	Hydrochloric acid treatment	Sodium carbonate fusion	Wet digestion	Individual laboratory method
1	17.2	17.6	18.6	—
2	12.7	12.3	—	—
3	10.0	12.0	—	13.3
4	—	—	—	16.5
6	13.6	14.2	16.8	13.2
7	12.4	12.0	12.1	—
8	15.8	16.8	13.5	13.9
10	12.8	13.8	13.3	13.9
11	12.3	12.4	—	12.3
12	13.0	13.6	—	—
13	10.2	14.8	—	—
14	13.8	16.2	—	—
15	14.6	15.0	14.8	—
16	—	—	—	18.1
17	—	—	—	13.9
Maximum	17.2	17.6	18.6	18.1
Minimum	10.0	12.0	12.1	12.3
Mean	13.2	14.2	14.8	14.4
Standard deviation	2.05	1.92	2.43	1.92

TABLE III

IRON CONTENT OF BREAD BAKED FROM ENRICHED FLOUR

Iron content as Fe in mg/lb ("as is" moisture basis—10.6%)

Collaborator number	Hydrochloric acid treatment	Sodium carbonate fusion	Wet digestion	Individual laboratory method
1	17.2	16.7	18.5	—
2	14.5	14.3	—	—
3	12.4	12.6	—	16.5
4	—	—	—	16.7
6	16.2	16.3	16.2	16.3
7	16.5	15.4	16.4	—
8	15.6	16.0	15.1	15.2
10	16.2	16.3	15.6	15.3
11	11.8	14.1	—	15.0
12	16.3	16.3	—	—
13	13.8	15.2	—	—
14	17.8	18.5	—	—
15	15.8	15.7	15.9	—
16	—	—	—	20.6
17	—	—	—	14.9
Maximum	17.8	18.5	18.5	20.6
Minimum	11.8	12.6	15.1	14.9
Mean	15.3	15.6	16.3	16.3
Standard deviation	1.85	1.49	1.18	1.87

TABLE IV

IRON CONTENT OF WHOLE WHEAT FLOUR

Iron content as Fe in mg/lb ("as is" moisture basis—12.6%)

Collaborator number	Hydrochloric acid treatment	Sodium carbonate fusion	Wet digestion	Individual laboratory method
1	18.9	18.1	18.4	—
2	16.1	14.8	—	—
3	14.0	13.3	—	17.2
4	—	—	—	18.5
6	16.3	16.3	14.3	16.7
7	16.4	16.0	18.1	—
8	17.0	17.5	16.6	16.3
10	18.6	16.7	18.0	16.0
11	14.5	18.2	—	19.1
12	18.5	17.0	—	—
13	16.3	14.1	—	—
14	19.0	18.6	—	—
15	17.6	17.5	17.0	—
16	—	—	—	22.7
17	—	—	—	16.9
Maximum	19.0	18.6	18.4	22.7
Minimum	14.0	13.3	14.3	16.0
Mean	16.9	16.5	17.1	17.9
Standard deviation	1.65	1.69	1.52	2.20

TABLE V

IRON CONTENT OF BREAD BAKED FROM WHOLE WHEAT FLOUR

Iron content as Fe in mg/lb ("as is" moisture basis—12.8%)

Collaborator number	Hydrochloric acid treatment	Sodium carbonate fusion	Wet digestion	Individual laboratory method
1	17.2	18.1	17.8	—
2	18.8	19.4	—	—
3	14.6	15.2	—	19.5
4	—	—	—	19.9
6	20.7	19.9	18.2	20.7
7	20.2	20.9	20.5	—
8	19.6	19.1	18.9	19.8
10	20.9	20.7	21.7	18.9
11	18.6	20.4	—	20.9
12	20.1	20.4	—	—
13	18.4	19.7	—	—
14	21.4	23.8	—	—
15	20.4	20.0	19.4	—
16	—	—	—	25.1
17	—	—	—	17.9
Maximum	21.4	23.8	21.7	25.1
Minimum	14.6	15.2	17.8	17.9
Mean	19.2	19.6	19.4	20.3
Standard deviation	1.90	1.99	1.47	2.15

TABLE VI

AVERAGE RESULTS OF THE COLLABORATORS WHO EMPLOYED ALL THREE METHODS

Iron content as Fe in mg/lb ("as is" moisture basis)

Sample	Hydrochloric acid treatment	Sodium carbonate fusion	Wet digestion
(1) Patent flour	3.9	4.0	4.4
(2) Enriched patent flour	14.4	14.9	14.9
(3) Bread made from enriched patent flour	16.3	16.1	16.3
(4) Whole wheat flour	17.4	17.0	17.1
(5) Bread made from whole wheat flour	19.8	19.8	19.4

Acknowledgment

The members who collaborated in this study were A. Wahl, E. F. Budde, Joseph Rosin, Aaron Arnold, John Andrews, C. G. Harrel, C. N. Frey, Wendell Reeder, Charles Hoffman, M. H. Neustadt, Morris Mead, Oscar Skovholt, Rosaltha Sanders, John B. Thompson, Jr., and Clarence Felt.

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REPORT OF 1943–44 COMMITTEE ON SELF-RISING AND PHOSPHATED FLOURS

ELIZABETH MCKIM, Chairman

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The formula as given in the Biscuit Test for Self-rising Flour (Cereal Laboratory Methods, 4th ed., 1941, p. 122) is based on soft wheat flour and the work of this year's committee was the adaptation of this test to hard wheat flour.

Hard wheat flours differ from soft wheat flours in that they require more water to make a soft dough and do not give as tender a biscuit. The problem then was to determine the proper absorption to use and whether the amount of shortening should be increased. The flours chosen for this test were a soft wheat flour and a hard wheat flour, both of family grade, the soft wheat flour to be used as the standard. These were about 80% patents and analyzed as follows:

	Soft wheat flour	Hard wheat flour
Moisture	15.0%	15.0%
Ash	0.36%	0.46%
Protein	8.1%	10.0%
pH	5.1	6.0

The flours were plain, and each collaborator was asked to make them into self-rising flour, using either of the following formulas:

	Monocalcium phosphate	
	Hydrated	Anhydrous
	g	g
Flour (15% moisture basis)	227.7	227.70
Sodium bicarbonate	3.4	2.85
Phosphate	4.3	3.42
Flour salt	4.6	4.69

Biscuits were baked from the self-rising soft wheat flour, following the formula and procedure given in the aforementioned Biscuit Test for Self-rising Flour. These biscuits were used as the reference against which the other biscuits were scored.

The hard wheat self-rising flours were baked using 12.5, 15.0, and 17.5% shortening (basis self-rising flour at 15% moisture). As the shortening is increased, it is necessary to decrease somewhat the amount of water used. Preliminary bakings showed that for each 2.5% increase in shortening the absorption should be decreased 1%. This agrees with the data of Schwain and Loving.[1] These preliminary bakings also indicated that the absorption of this hard wheat self-rising flour (15% moisture and 12.5% shortening) was 66.6%. Several of the collaborators found this to be about 1% too high.

[1] Schwain, F. R., and Loving, H. J., 1944. The shortener tolerance of biscuit and self-rising flours. Cereal Chem. 21: 27–32.

Six members of the committee collaborated on the bakings and the average scores are shown in Table I. The variations in the individual scores were so great that the averages shown in the table mean very little. However, from the conclusions reached by each member, definite recommendations can be made. Four of the collaborators

TABLE I

EFFECT OF INCREASING FAT IN BISCUIT FORMULA USING HARD WINTER WHEAT FLOUR [1]

Factor	Perfect score	Scores of biscuits made by six collaborators					
		12.5% fat		15% fat		17.5% fat	
		Average	Range	Average	Range	Average	Range
Grain	10	8.4	7–10	9.0	7.7–10	9.1	8.0–10
Tenderness	10	8.3	7.7–9.0	9.1	8.7–9.4	9.5	9.0–9.7
Flavor	20	19.0	18.0–20.0	19.3	18.5–20.0	18.8	17.0–20.0
Color	20	17.4	15.0–19.1	17.8	15.7–19.5	17.5	16.0–19.3
Volume	40	40.6	38.0–43.3	39.8	39.0–41.4	38.6	34.0–43.1
Total	100	93.8	90.2–96.2	95.0	91.4–98.8	93.4	89–97.8
pH		7.08	6.71–7.28	7.08	6.71–7.27	7.15	6.71–7.27

[1] Cereal Laboratory Methods, 4th ed., 1941.

Four of the collaborators preferred the biscuits made with 15% shortening, while two preferred those made with 17.5% shortening.

thought that 15% shortening gave optimum results, while the other two thought that 17.5% fat could be used. Two of the four who got the best results with 15% fat got definitely poorer biscuits when the amount was increased to 17.5%. Absorption values checked fairly well, varying from 62.5% to 65.6% where 15% shortening was used and the self-rising flour moisture was 15%.

On the basis of these conclusions the committee recommends the following formula for the Biscuit Test for Self-rising Flour made with hard winter wheat flour:

Ingredients	Grams	Percentage based on flour	Percentage composition
Flour, hard winter self-rising (15% moisture basis)	240	100	55.6
Shortening, hydrogenated (25°C)	36	15.0	8.4
Water, distilled	155	64.6	36.0
Totals	431		100.0

Acknowledgments

The members who collaborated in this study were: R. A. Barackman, V. E. Fisher, H. R. Goforth, Elizabeth McKim, R. M. McKinstrie, W. C. Meyer, and Elmer Modeer.

NUTRIENT CONTENT OF ALCOHOL FERMENTATION BY-PRODUCTS FROM VARIOUS GRAINS

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(Received for publication June 5, 1944)

The nutrient content of alcohol fermentation by-products from yellow corn grain mixtures as produced under commercial conditions has been determined recently by Bauernfeind, Garey, Baumgarten, Stone, and Boruff (1944). These corn grain mash bills (formulae) are favored for the production of whiskey or alcohol, and the recovery of by-products from them has been in-process for many years. During the past year certain sections of the country have experienced an apparent corn shortage, and the distilling industry has been forced to use other grains such as wheat, granular wheat flour, and rye. The use of these other grains has raised some questions as to the composition and nutritive value of by-products produced from them. It is the purpose of this paper to present data on the nutrient content of these by-products prepared with pilot plant equipment.

Experimental Fermentations

A weighed quantity of corn, wheat, granular wheat flour, or rye was cooked in pilot plant equipment at maximum temperatures of 155°, 212°, 280°, and 312°F (68.3°, 100.0°, 137.8°, and 155.6°C) respectively. The amounts of water used per bushel of grain were as follows: 20 gal for corn cooked at any of the specified temperatures, and for wheat and granular wheat flour cooked at 155°F (68.3°C); 13.8 gal for wheat and granular wheat flour cooked at each of the specified temperatures above 155°F and for rye cooked at 280°F (137.8°C); and 22 gals for rye cooked at 155°F (68.3°C). After cooking and cooling to malting temperature, 140–146°F (60.0–63.3°C), barley malt was added for the purpose of conversion. The mash bills for this report were (1) wheat 92%, barley malt 8%; (2) granular wheat flour 92%, barley malt 8%; (3) rye 90.9% and barley malt 9.1%; (4) yellow corn 90.9%, barley malt 9.1%. After conversion, the mashes were cooled and inoculated with 3% by volume of yeast from the regular plant yeast and set at 40 gal of mash per bushel of air-dry grain. The fermentations were incubated at 90°F usually for 72 hr. The alcohol yield in proof-gal per

[1] Present address: Hoffman-LaRoche, Inc., Nutley, New Jersey.

56-lb bushel of moisture-free grain was 5.2–5.5 for wheat, 5.6–6.0 for corn, 4.7–5.1 for rye, and 6.3–6.5 for granular wheat flour.

Pilot Plant By-Product Recovery Equipment

A pilot plant recovery system of a suitable size was planned and put into operation to process the stillage from experimental 5-gal fermentations. The system involved the following equipment:

1. Metal screen with a drainage pan, constructed at a 15° angle, 36 inches long, 8 inches wide, with screen openings 1 mm in diameter on 0.075-inch centers.
2. Batch vacuum evaporators, 5- and 12-gal capacity, steam-heated.
3. Atmospheric double-drum dryer, maximum steam pressure 160 lb per sq inch; surface area of each drum 1 sq. ft.
4. Drying rack with vertical adjustment holding several infra-red drying bulbs.

By-Product Recovery

The recovery procedure in the pilot plant operations was the same in all cases. A weighed quantity of beer (approximately 4 gal) was distilled under vacuum for one hr at a vapor temperature of 145–155°F (62.8–68.3°C) to remove the alcohol. Steam was then passed into the beer to bring it to a temperature range of 208–212°F (97.8–100.0°C). The hot stillage was adjusted to the weight of the original beer by adding water and was immediately screened. The rate of flow over the screen was controlled by use of a hand-operated wooden baffle. The grains (screenings) were manually pressed on the screen to remove most of the absorbed liquid. The weighed screen effluent was concentrated at 160–165°F (71.1–73.9°C) to a syrup of approximately 20% total solids. The syrup was converted to dried solubles on the drum dryer operating at 140–150 lb steam pressure, with a drum clearance of 0.015 inches and with a drum rotation rate of 2.75 rpm. As judged by appearance and odor, a satisfactory product was produced from all fermentations. The pressed distillers' grains were dried under infra-red lamps.

Corn stillage screened most easily, while granular wheat flour stillage screened very slowly. The other two were intermediate, with wheat stillage showing slightly greater screening ease than rye. The moisture-free total by-product yield per 56-lb bushel of air-dried grain was 18.5–20 lb for wheat, 16–17.5 lb for corn, 20–21.5 lb for rye, and 15–15.5 lb for granular wheat flour. The amounts of distillers' solubles and distillers' grains were about equal for wheat; the grains slightly exceeded the solubles for corn; the solubles slightly exceeded the grains for rye; and for granular wheat flour, the amount of solubles more than doubled the amount of distillers' grains. Definitions for the distillers' by-products have been given by the Association of American Feed Control Officials (1944).

In order to ascertain whether the pilot plant screening and pressure procedure would yield a product comparable to that obtained in commercial operations, samples of whole stillage were taken from plant operations and screened in the pilot plant equipment. These grains (screenings) are compared with the regular commercial products in Table I. The data show the pressed distillers' grains from the two operations to be quite comparable.

TABLE I

ANALYSES OF PRESSED DISTILLERS' GRAINS PREPARED DURING PILOT PLANT AND COMMERCIAL OPERATIONS [1]

Trial	Process	Solids content	Proximate composition (dry-matter basis)			
			Protein	Fat	Fiber	Ash
		%	%	%	%	%
1	Pilot plant	23.9	31.1	9.2	12.9	2.1
	Commercial	26.2	31.4	10.1	13.8	1.9
2	Pilot plant	24.3	32.5	8.8	13.2	2.2
	Commercial	26.7	31.0	9.6	14.2	2.3
3	Pilot plant	25.5	30.9	11.4	13.0	2.1
	Commercial	26.7	31.0	11.3	13.1	2.0
4	Pilot plant	24.7	31.5	11.9	14.1	2.3
	Commercial	27.4	30.8	9.6	15.3	1.9

[1] The mash bill employed in the above study contained the following grains:

Corn	—28.13%
Wheat	—48.58%
Granular wheat flour	—10.56%
Barley malt	— 9.93%
Rye	— 2.80%

Methods of Analysis

Moisture-free samples were obtained by drying at 105°C to constant weight. All protein data in the paper are nitrogen values multiplied by 6.25. Thiamine was determined by the thiochrome method of Hennessy (1941). Riboflavin was assayed by the method of Snell and Strong (1939). Samples were extracted with 0.1*N* HCl in an autoclave for 15 min at 15 lb pressure. Samples for the pantothenate assay were extracted with water at a pH of 6.5–6.8 for 15 min after which the microbiological method of Pennington, Snell, and Williams (1940) was followed. Crystalline d-calcium pantothenate served as the reference standard. Care was taken in both these assays to avoid the growth stimulants described by Bauernfeind, Sotier, and Boruff (1942). The Snell and Wright (1941) method for nicotinic acid was employed and the samples were extracted with 0.1*N* NaOH for 15 min in an autoclave.

Experimental Results and Discussion

The proximate feed analyses and mineral composition of the by-products are presented in Tables II and III, respectively. As the cooking temperature of the grains was increased, a greater percentage of protein was found in the distillers' grains (screenings). Heat has

TABLE II

CHEMICAL COMPOSITION OF ALCOHOL FERMENTATION BY-PRODUCTS FROM VARIOUS GRAINS

Fermentation by-product	Cooking temperature [1]		Proximate composition (dry-matter basis)			
			Protein	Fat	Ash	Fiber
	°C	°F	%	%	%	%
Wheat distillers' dried solubles	68.3	155	46.5	0.5	8.8	2.2
	100.0	212	39.0	0.8	10.1	2.5
	137.8	280	37.0	0.9	10.0	2.4
	155.6	312	35.8	0.6	10.1	2.3
Wheat distillers' dried grains	68.3	155	27.2	5.5	2.3	14.7
	100.0	212	37.9	4.7	2.8	12.0
	137.8	280	39.5	4.3	2.5	13.1
	155.6	312	42.6	—	2.1	13.9
Corn distillers' dried solubles	100.0	212	21.7	5.2	9.7	2.9
	137.8	280	28.5	6.6	9.5	3.8
	155.6	312	24.7	—	9.3	3.1
Corn distillers' dried grains	100.0	212	30.5	8.3	1.9	12.1
	137.8	280	32.3	10.3	2.0	12.8
	155.6	312	33.9	11.4	1.8	13.2
Rye distillers' dried solubles	68.3	155	40.4	0.7	8.0	2.6
	137.8	280	36.4	0.7	8.5	2.8
Rye distillers' dried grains	68.3	155	24.0	6.3	2.2	13.0
	137.8	280	28.9	6.8	2.3	13.4
Granular wheat flour distillers' dried solubles	68.3	155	46.0	1.1	5.3	2.2
	137.8	280	42.8	1.3	4.9	2.9
Granular wheat flour distillers' dried grains	68.3	155	27.0	4.5	2.6	15.7
	137.8	280	44.0	3.2	2.4	11.2

[1] Refers to maximum temperature reached in the cooking cycle.

long been recognized as a method of denaturing proteins and this makes them more insoluble. Once the protein is precipitated it is more easily retained among the other coarser grain residues as they pass over the screen. Conversion of the starch to alcohol and carbon dioxide results in a concentration of the remaining nutrients of the original grain in the composite by-products of fermentation. Conversely then, from the data on the composition of the by-products and with the use of

quantitative by-product recovery data, the analyses of the original cereal mixture grain can be computed as a check on the by-product analyses. For example, when this is carried out on the wheat by-products the computations show the following composition for the wheat mixture (92% wheat, 8% barley malt) on an air-dry basis: protein 13.1%, fat 1.9%, fiber 2.7%, ash 2.1%, calcium 0.12%, and

TABLE III

MINERAL COMPOSITION OF ALCOHOL FERMENTATION BY-PRODUCTS FROM VARIOUS GRAINS

Fermentation by-product	Cooking temperature [1]		Calcium [2]	Phosphorus [2]
	°C	°F	%	%
Wheat distillers' dried solubles	137.8	280	0.52	1.70
Wheat distillers' dried grains	137.8	280	0.22	0.50
Corn distillers' dried solubles	155.6	312	0.51	1.50
Corn distillers' dried grains	155.6	312	0.15	0.24
Rye distillers' dried solubles	137.8	280	0.46	1.48
Rye distillers' dried grains	137.8	280	0.13	0.37
Granular wheat flour distillers' dried solubles	137.8	280	0.48	0.91
Granular wheat flour distillers' dried grains	137.8	280	0.20	0.35

[1] Refers to maximum temperature reached in the cooking cycle.
[2] Dry-matter basis.

phosphorus 0.38%. Computations from the rye by-products show the following composition for the rye mixture (90.9% rye, 9.1% barley malt) on an air-dry basis: protein 12.0%, fat 1.3%, fiber 2.7%, ash 2.0%, calcium 0.11%, and phosphorus 0.38%. In both cases it will be noted that the analyses are in fairly good agreement with accepted values for the mixtures of cereal grains, with the exception of the calcium content. Usually these grains contain 0.04–0.06% calcium. The difference between these values and the 0.11–0.12% obtained from the above computations is attributed to the calcium supplied by the use of limestone-bearing well water in the process. Boruff, Smith, and Walker (1943) reported the calcium content of limestone-bearing well water to be 125.7 ppm. About 33 to 38 gal of water are used per bu of grain fermented, supplying the additional 0.06–0.07% calcium.

Thiamine, riboflavin, nicotinic acid, and pantothenate values for the various by-products are reported in Tables IV and V. In the case of the corn and wheat distillers' dried solubles, a higher thiamine content was observed when they were prepared from grain mashes cooked at 212°F than at 312°F, which indicates that greater destruction of thiamine was caused by the higher cooking temperature. The riboflavin,

TABLE IV

VITAMIN COMPOSITION OF ALCOHOL FERMENTATION BY-PRODUCTS FROM VARIOUS GRAINS

Fermentation by-product	Cooking temperature [1]		Vitamin content (dry-matter basis)			
			Thiamine	Riboflavin	Nicotinic acid	Pantothenate
	°C	°F	μg/g	μg/g	μg/g	μg/g
Wheat distillers' dried solubles	68.3	155	—	15.2	210.0	—
	100.0	212	10.0	13.0	232.0	44.0
	137.8	280	4.5	14.0	228.0	46.5
	155.6	312	4.2	14.1	220.0	45.0
Wheat distillers' dried grains	68.3	155	—	4.1	72.0	7.8
	100.0	212	1.8	3.3	86.0	7.4
	137.8	280	1.8	3.7	82.0	7.0
	155.6	312	1.3	2.9	74.0	6.3
Corn distillers' dried solubles	100.0	212	11.0	17.8	172.0	25.0
	137.8	280	6.5	15.1	141.0	25.5
	155.6	312	7.9	18.6	162.0	22.0
Corn distillers' dried grains	100.0	212	2.2	3.5	47.0	4.4
	137.8	280	1.5	3.3	31.0	5.0
	155.6	312	1.5	4.0	43.0	3.9
Rye distillers' dried solubles	68.3	155	4.0	13.2	45.0	33.0
	137.8	280	3.5	14.0	52.0	35.0
Rye distillers' dried grains	68.3	155	1.9	3.6	18.0	7.0
	137.8	280	1.6	3.8	19.0	5.5
Granular wheat flour distillers' dried solubles	68.3	155	5.3	11.5	81.0	23.7
	137.8	280	6.0	10.9	80.0	24.3
Granular wheat flour distillers' dried grains	68.3	155	2.6	3.4	42.5	5.9
	137.8	280	2.2	4.5	46.8	4.4

[1] Refers to maximum temperature reached in the cooking cycle.

TABLE V

VITAMIN CONTENT [1] OF COMPOSITE FERMENTATION BY-PRODUCTS

Fermentation by-product	Thiamine	Vitamin content (dry-matter basis)		
		Riboflavin	Nicotinic acid	Pantothenate
	μg/g	μg/g	μg/g	μg/g
Wheat distillers' dried grains with solubles	3.1–5.9	8.8	150.0	26.1
Corn distillers' dried grains with solubles	4.1–6.3	9.9	94.1	13.5
Rye distillers' dried grains with solubles	3.0	8.9	34.4	21.0
Granular wheat flour distillers' dried grains with solubles	4.5	8.8	68.5	18.7

[1] Calculated from Table 4 using quantitative by-product recovery data.

nicotinic acid, and pantothenate contents were not significantly affected by the various cooking procedures.

The vitamin contents of the fermentation by-products were determined, computed in terms of the original grain mixtures, and compared with the vitamin content found by direct analyses of the original grain mixtures in order to indicate whether the vitamin values of the by-products represented a simple concentration of vitamins during processing, a synthesis of vitamins by microorganisms, or a partial destruction of the vitamins resulting from the heat treatment. This computation, as well as the vitamin content of the original grain bill as found by assay or by use of literature values, is shown in Table VI.

TABLE VI

VITAMIN CONTENT OF EXPERIMENTAL MASH BILLS

Mash bill	Method of estimating vitamin content [1]	Vitamin content (air-dry basis)			
		Thiamine	Riboflavin	Nicotinic acid	Pantothenate
		μg/g	μg/g	μg/g	μg/g
92% wheat and 8% barley malt	A	1.0–2.0	3.0	51.0	8.9
	B	4.2	1.1	45.0	10.8
90.9% corn and 9.1% barley malt	A	1.2–1.9	3.0	28.2	4.0
	B	3.6	1.2	27.7	6.3
90.9% rye and 9.1% barley malt	A	1.1	3.3	12.7	7.7
	B	2.9	1.3	16.0	9.8
92% granular wheat flour and 8% barley malt	A	1.3	2.4	19.0	5.3
	B	2.2	0.6	22.8	6.2

[1] Method A: Assay values for by-products, divided by factor of concentration (56 lb original grain ÷ no. of lb recovered material).
Method B: Values obtained by vitamin assay of the original cereal grains in the laboratory and from the 1942 and 1943 literature on this subject.

It will be noted that some thiamine and some pantothenate originally present in the grains have apparently been destroyed during processing. There is no consistent increase or decrease in nicotinic acid content on the basis of this comparison, and hence it is concluded that the content of this vitamin in these fermentation by-products is due primarily to a concentration of that present in the original cereal grains. On the other hand, the riboflavin content is significantly greater than can be accounted for by the amount present in the original grains, and the additional amount of vitamin must be attributed to the synthesizing ability of the microorganisms.

There are several periods during the production of these fermentation by-products when the material is subjected to heat. The first,

which was a variable in this report, is the cooking of the grain. While the maximum cooking temperature was maintained for only 15 min, the entire heating period for the mash to reach the maximum temperature and to cool to and below the malting temperature, required about 1.5 hr. During this time the pH was 5.5–6.2. The results in Table IV show that more thiamine was destroyed upon cooking to a temperature of 312°F (155.6°C) than to 212°F (100°C).

In the evaporation of the screen effluent during the pilot plant study, a temperature of 160–165°F (71.1–73.9°C) was applied for approximately 6 hr. The pH of the screen effluent was 3.8–4.1. In the commercial process, only 4 to 5 hr are required, and the temperature varies from 220°F (104.4°C) in the first evaporator to 130°F (54.4°C) in the finishing pan. In the drum-drying of the concentrated syrup the product is subjected to a high temperature for a short period of time, about 20 sec, and usually there is little or no destruction of pantothenate in this time. If the knife blades are raised on the drum and the product permitted to remain there for 100 sec, about 50% of the pantothenate present in the syrup is destroyed. The available evidence at the moment shows no loss of riboflavin or nicotinic acid during the recovery of the by-products.

Beadle, Greenwood, and Kraybill (1943), in studying the stability of thiamine to heat, reported that it was a function not only of pH but also of the electrolyte system. At a pH of 5.4 and at boiling temperature for 1 hr, 57% of the thiamine in pure water was destroyed, while only 10% was destroyed in a dilute acetate buffer. Destruction was greater at pH values higher than 5.4. Frost (1943) found the rate of destruction of pantothenate to be a function of pH and temperature and also to be affected by other substances in aqueous solution. He found that if a 1% dextrorotatory calcium pantothenate aqueous solution at pH 4.0 was stored at 60°C for 5 days that 26% of the activity was destroyed, while in a dilute phosphate buffer solution 10% was destroyed.

Synthesis of riboflavin by microorganisms, bacteria, yeast, and molds has been reported by a number of investigators. Hence riboflavin synthesis does not seem unlikely here. Laufer, Davis, and Saletan (1942) assayed the raw materials, the intermediate, and the end products of both an ale and a lager brew for vitamin content to determine their fate in the brewing process. On the basis of the data for the products assayed, the authors concluded that there is some loss of thiamine in the brewing process due to destruction of the vitamin, that there is relatively little loss or gain of nicotinic acid, and that a possible synthesis of pantothenate and riboflavin by yeast is indicated.

Summary

The proximate, mineral, and vitamin compositions of fermentation by-products in the production of alcohol from corn, wheat, granular wheat flour, and rye have been presented. As the cooking temperature of the cereal grains was increased, a greater percentage of the protein in the total by-products was found in distillers' grains (screenings). The use of limestone-bearing water in the process significantly increased the calcium content of the by-products. In the production of the fermentation by-products, some thiamine and pantothenate were destroyed, presumably by heat treatment at sensitive pH levels. The nicotinic acid content of the by-products was due primarily to a concentration of the nicotinic acid present in the original grains, while the riboflavin content of the by-product was derived both from the riboflavin in the original grains and from synthesis by microorganisms.

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PRODUCTION OF STARCH FROM WHEAT AND OTHER CEREAL FLOURS

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(Received for publication May 13, 1944)

Normally about 98% of the starch production in the United States (approximately 2,500 million pounds annually) is accounted for by the wet milling of corn. Since 1940 starch production has increased, the wet-milling industry having processed 130 million bushels of corn in 1942 compared with about 75 million bushels annually prior to 1940. The recent shortage of corn on the cash market, coupled with the increased demand for starch and its conversion products for use in food and essential industries, has directed attention toward the use of other cereal grains for starch production. Wheat, in particular, has received consideration as an alternative source of starch, the fermentation industry having turned to it in 1942 as a partial replacement for corn in alcohol production. The utilization of wheat and other cereal grains as raw material for maintaining or increasing starch production should be based on processing methods which permit: (a) production of starch of low protein content in good yields for use as such or for fermentation or conversion to glucose sirup or sugars, (b) recovery of by-product protein, (c) adaptation to existing plant facilities with a minimum of new installations involving critical materials, and (d) use of different varieties of wheat and other cereal grains if required.

Wheat is the earliest recorded material from which starch was prepared. The technical and patent literature on starch production, however, is largely confined to the use of corn for wet-milling operations. In some cases applicability of the steps to wheat and other grains is claimed, although there is little or no evidence of actual practice on these grains. The two well-known processes for the production of starch from wheat grain are the Halle or fermentative process and the Alsatian or nonfermentative process. In these processes a water steep with subsequent wet milling is employed. Recent studies by Slotter and Langford (1944) have demonstrated the applicability of modern corn wet-milling practice to wheat grain. Such a process would be suited to the utilization of wheat in existing wet-milling plants when desired.

Existing wheat starch manufacturers have generally found it advantageous to use flour as the raw material. Wheat flour has a

[1] This is one of four regional research laboratories operated by the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

higher starch content than the grain, can be processed more rapidly with less plant space and equipment, and its use minimizes by-product recovery, since most of the bran and fiber is retained at the flour mill. The main disadvantage of the use of flour is its higher cost on an equivalent starch basis.

The most generally used method, at present, for producing starch from wheat flour is the Martin process (Eynon and Lane, 1928), based on washing the starch out of a dough. This process provides ready recovery of by-product wheat gluten and a good yield of starch. The Martin process, however, is not readily adaptable to large-scale operation, is limited in applicability to wheat flour having good doughing properties, and cannot be used to separate starch from other cereal flours. Modifications of the Martin process have been suggested (Eynon and Lane, 1928) which eliminate the dough-washing step by substitution of a centrifugal separation of the gluten and starch from a dough or paste. These methods, however, do not effect a clean-cut separation of starch and protein.

In addition to these primarily mechanical procedures for wheat starch production, methods largely dependent on chemical treatment, particularly with alkali, have been proposed. Alkali disperses or dissolves the protein, thereby facilitating separation of the starch in a state of high purity. Jones (1841) patented a process for the separation of starch from rice or wheat meal by an alkali treatment which is still used as the basis for rice starch production. However, no detailed information is available in the literature on the use of alkali in the production of starch from wheat, wheat flour, or other cereal grains except rice and corn industrially.

This paper deals with an investigation of processing conditions and methods which might be used for preparing starch and protein from flour by alkali treatment. Some information is available in the literature on the action of alkali on wheat protein and wheat flour. Relative to the problem of starch production these data are incomplete. The present studies therefore may be resolved into two sections: (a) establishment of background information on the behavior of the starch and protein in wheat flour in the presence of alkali, and (b) application of this knowledge to the preparation of starch and by-product protein.

Experimental Methods and Results

Flours Used. Most of the flour samples from wheat and other cereal grains used for these studies were prepared in an experimental Buhler flour mill using 10XX bolting silk (110 mesh). With Rex soft white wheat and Fulton oats the tendency of the flour to "ball-up" on the fine silk necessitated the use of a No. 48 or No. 64 grit gauze

(50 to 60 mesh) as the finest bolting cloth in the system. The samples of granular and second clear wheat flour and white rye flour were commercially prepared products. The starch and protein content and the milling yield of the various flour samples used are shown in Table I.

TABLE I

ANALYSES OF FLOUR SAMPLES

(All data on moisture-free basis)

Kind of flour	Milling yield	Starch content	Protein content
	%	%	%
No. 2 Dark Northern Spring wheat (from Commodity Credit Corporation)	70	78	13.7
Thatcher hard red spring wheat	72	76	16.4
No. 2 Hard Winter wheat (from Commodity Credit Corporation)	70	80	12.7
American Banner soft white wheat	70	81	10.6
Rex soft white wheat	74	81	7.0
Second clear (hard red spring wheat) [1]	3	64	20.9
Granular wheat (grits) [1]	60	77	12.9
White rye [1]	55	76	9.6
Trebi barley	45	79	8.4
Fulton oats	62	72	13.0
Illinois Hybrid No. 972 corn	21	79	7.5
Pink kafir (sorghum)	33	88	6.9
Colusa rice	59	92	4.7

[1] Samples commercially prepared.

The protein value, based on Kjeldahl nitrogen, was calculated as $N \times 5.7$ for all except corn and sorghum, for which the factor 6.25 was used. The starch was determined polarimetrically by a modification (Clendenning, K. A., private communication) of the Hopkins' procedure (1934).

Dispersing Action of Alkalies on Wheat Protein. The dispersing action of aqueous alkalies on wheat protein was determined by a modification of the procedure for the determination of water-soluble protein nitrogen according to A.O.A.C. (1940). Twenty g of flour was mixed with 200 ml of the alkali solution and shaken frequently for 30 min. The mixture was then allowed to settle for 2 hr and the nitrogen content of the supernatant was determined. The apparent protein solubility, expressed in percent, was calculated as follows:

$$\text{Apparent protein solubility} = \frac{\text{mg } N \text{ per ml supernatant} \times 200}{\text{mg } N \text{ per g flour} \times 20} \times 100.$$

This represents the percent of the total protein which is dispersed, under the conditions used, to such a degree that it does not readily settle by gravity.

Measurements were made of the protein-dispersing power of solutions of sodium, potassium, calcium, and barium hydroxide and of sodium carbonate, using American Banner soft white wheat flour. The observed apparent protein solubilities, given in Table II, indicate

TABLE II

DISPERSION OF PROTEIN IN AMERICAN BANNER WHEAT FLOUR BY VARIOUS ALKALIES

Alkali	Apparent solubility	pH of mixture [1]
	%	
NaOH 0.03*N*	101	11.8
KOH 0.03*N*	100	11.7
NaOH 0.01*N*	95	10.9
KOH 0.01*N*	98	10.8
$Ca(OH)_2$ 0.05*N*	92	11.7
$Ba(OH)_2$ 0.05*N*	90	11.7
Na_2CO_3 0.3%	66	10.3

[1] Measured with glass electrode, uncorrected for alkali salt errors.

that maximum removal of protein from wheat flour is effected by sodium or potassium hydroxide. If a higher protein content in the starch can be tolerated, the use of calcium hydroxide offers the advantage of low raw material cost. Sodium hydroxide was used for most of the studies dealing with the effect of various factors on the production of starch with a low protein content.

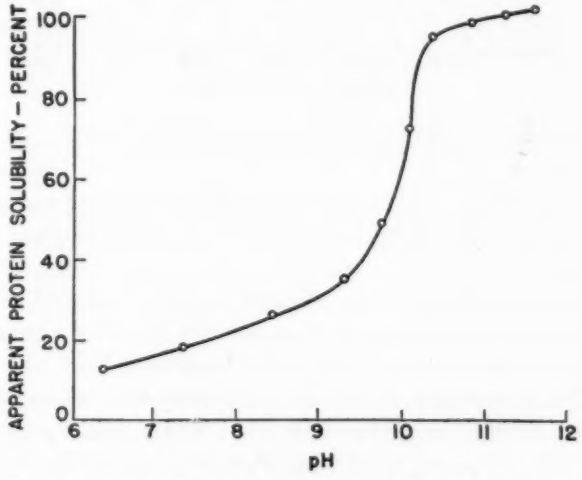

Fig. 1. Relationship of the extent of wheat protein dispersion to the pH of the mixture of flour and aqueous sodium hydroxide.

The relationship between the apparent protein solubility and the pH of the mixture of aqueous sodium hydroxide and No. 2 Hard Winter wheat flour (12.6% protein, moisture-free basis) is shown in Figure 1.

For the flour from other varieties of wheat and from rye and barley it was found that the use of 0.01*N* NaOH resulted in a pH of 10.4 to 10.8 and an apparent protein solubility of about 95%, while 0.03*N* NaOH gave a pH of about 11.4 to 11.7 and essentially complete protein dispersion. For any given flour the pH of the mixture appeared to be primarily dependent on the ratio of flour to sodium hydroxide and influenced only to a minor extent by variations of the amount of water within practicable limits. The results show that, for maximum dispersion of the flour protein, the mixture of flour and sodium hydroxide should have a pH above 10.5.

The dispersion of the protein in the flour was very rapid in sodium hydroxide solutions. The shortest period for which a measurement was made was 10 min, in which time dispersion was complete. In

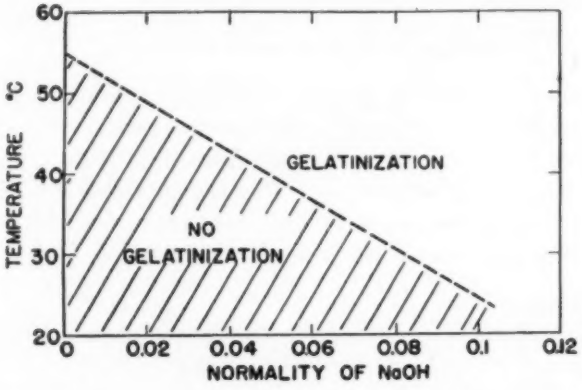

Fig. 2. Relationship of temperature and amount of sodium hydroxide to gelatinization of starch in wheat flour; 10 g flour per 100 ml of sodium hydroxide; 4-hr treatment.

pilot-plant operations, for which highly efficient mixing equipment was not available, periods of 15 to 30 min were usually allowed for the dispersion step.

Starch Gelatinization. Approximate working limits of temperature and sodium hydroxide concentration for the alkali treatment of flour without gelatinizing the starch were established in the following manner. Ten-g samples of American Banner soft white wheat flour were mixed with 100-ml portions of 0.1*N*, 0.05*N*, and 0.03*N* NaOH at about 25°, 30°, 40°, and 50°C and allowed to stand for 4 hr. Gelatinization was followed microscopically, using as an index the loss of birefringence and the staining by benzopurpurin of the granules. The approximate range of temperature and strength of sodium hydroxide in which little or no gelatinization of the starch occurred is shown by the shaded area in Figure 2. The pH was about 11.8 for

a mixture of the flour with 0.03*N* sodium hydroxide at room temperature.

Protein Recovery. Recovery of most of the protein was effected by acidifying the starch-free alkaline solution. The optimum pH for precipitating the protein was determined as follows:

An alkaline wheat protein dispersion was prepared by centrifuging the alkali-insoluble solids from a mixture of 100 parts (moisture-free basis) No. 2 Hard Winter wheat flour, 1,250 parts water and 1.5 parts NaOH (solid). Aliquots of this solution were adjusted to various pH values with H_2SO_4; the precipitated protein was separated by centrifuging; and the protein contents of the supernatants determined. The relation between pH and the percentage of protein precipitated is shown in Figure 3. Essentially the same results were obtained when

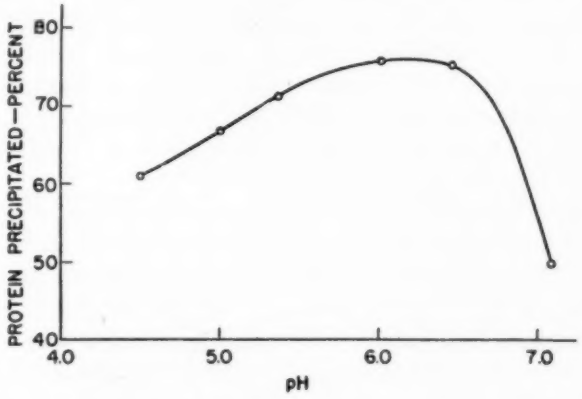

Fig. 3. Effect of pH on the extent of precipitation of wheat protein by acidification of the starch-free alkaline dispersion with sulfuric acid.

other acids, such as HCl, SO_2, and CO_2, were used, the lowest pH readily obtained with the last being about 6.3.

The flocculation and settling behavior of the protein was variable. At pH 6.0 and higher, frequent failures to flocculate and settle were encountered. At pH 5.5, however, flocculation occurred regularly with all of the wheat flours studied in this region of flour concentration. This has led to a preference for pH 5.5 for protein recovery in the procedures for processing flour despite the higher percentage of protein precipitated in the region of pH 6.0.

In the pH range of 5.0 to 6.0 the flocculated protein settled by gravity at a rate of between 1 and 2.5 ft per hr to occupy one half to one third of the total volume of fluid. Centrifugation at about 800 times gravity gave a soft protein cake containing 70 to 80% moisture. When dried, this cake became hard and brittle and had a protein

content of about 85% ($N \times 5.7$), moisture-free basis, representing from 67 to 77% of the protein originally present in the alkaline solution.

The protein precipitated by acidification was finely divided and did not filter readily. Evidence was obtained which confirmed the findings of the Overly Biochemical Research Foundation, Inc. (private communication, 1943), that the use of a lignin sulfonate as a precipitation aid might be effective in giving recovery of protein in a filterable form. The proportion of dry lignin sulfonate required, however, was undesirably high, being approximately equal in weight to the protein to be precipitated.

The concentration of protein in the alkaline solution was increased either by raising the flour concentration or, more practicably, by using the alkaline starch-free liquor from one flour dispersion for the treatment of additional portions of flour (with the necessary addition of alkali) before precipitating the protein. A concentration of protein and other alkali solubles was reached, however, at which the protein failed to flocculate and settle, even at pH 5.5. In qualitative experiments on wheat flour, such failure was encountered with an alkaline solution equivalent to that from the treatment of 1 part flour with 3 parts aqueous alkali.

The wheat protein recovered by precipitation from the alkaline dispersion had undergone some chemical change. When the solutions were acidified, the odor of hydrogen sulfide was noted, probably indicating changes involving cysteine. The recovered protein was no longer glutinous in character, although its solubility in solvents such as acetic acid or alcohol was not greatly different from that of "native" wheat gluten.

Processes for Starch Separation after Alkali Treatment of Flours. Two processes, centrifuging and tabling, were used for separating starch from flour after alkali treatment. The details of conditions and processing steps, based on the preceding data and on trial processing, were chosen with the objective of providing applicability to a wide variety of flours. To facilitate the separation of a maximum of protein from the starch, a pH above 10.6 was used, thus insuring essentially complete dispersion of the protein by sodium hydroxide (see Fig. 1). An upper limit of pH 11.8, obtained with about 1.5 parts NaOH per 100 parts flour, was set, along with an upper temperature limit of 35°C, to avoid the region of starch gelatinization shown in Figure 2. Exploratory experiments showed that increasing the flour concentration, although resulting in an economy in the volume of fluids, decreased the rate of settling of the starch and increased the amount of protein in the liquor held in the starch layer. For the tabling of starch from the alkaline wheat flour suspension, a concentra-

tion of 1 part flour in 12.5 parts of 0.03*N* NaOH was found most practical, while for centrifuging, 1 part flour in 6 parts of 0.03*N* NaOH could be used for most flours.

The two processes for starch and by-product protein production, as described below in detail, illustrate the use of representative conditions and processing steps. Considerable variation of the processes is possible depending on the ingenuity of the processor, the equipment used, and the purity required of the products. The results of the processing of different flours are given to indicate the applicability to a wide variety of raw materials.

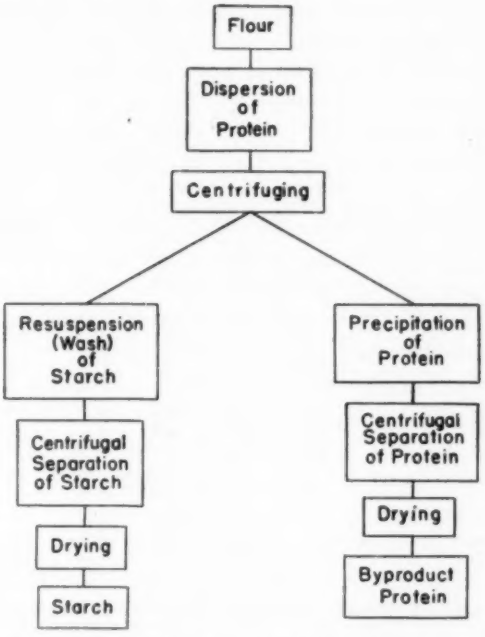

Fig. 4. Centrifuging process flow sheet for obtaining the products in Table III.

Centrifuging Process and Application to Various Wheat Flours. In the centrifuging process the starch is separated in a single fraction, together with the small amounts of other alkali-insoluble solids, for the production of a low-protein crude starch by a minimum number of operations. The general procedure adopted for the centrifugal separation of starch from alkali-treated flour is outlined in the flow sheet (Fig. 4).

For the preparation of starch, 150 g (100 parts) of flour (moisture-free basis) was mixed with 900 ml of 0.03*N* NaOH (600 parts aqueous solution containing 0.75 parts NaOH) at between 25° and 35°C with

rapid stirring until all lumps of flour had disintegrated, usually within 15 min. This flour suspension (about 8.0° Baumé) was introduced into an imperforate basket centrifuge at such a rate that the liquor overflowing the rim of the basket was starch-free. The cake of crude starch was resuspended in about 800 ml of water with vigorous stirring, and the suspension introduced into the centrifuge basket as before, without neutralization. The resulting starch was dried at 40°C in a mechanical convection oven. When a neutralized starch was desired, the washed starch was resuspended and the pH adjusted.

TABLE III

YIELD OF STARCH AND PROTEIN FROM VARIOUS FLOURS—CENTRIFUGING PROCESS

(All data calculated on moisture-free basis)

Kind of flour	Crude starch	Washed starch [1]			Precipitated protein		
	Protein content	Yield	Starch recovery	Protein content	Yield	Protein content	Protein recovery
	%	*Lb*/100 *lb flour*	%	%	*Lb*/100 *lb flour*	%	%
Rex soft white wheat	0.9	86	101	0.3	6	70	63
No. 2 Hard Winter wheat	1.7	83	101	0.4	10	87	71
No. 2 Dark Northern Spring wheat [2]	2.7	82	100	0.4	11	85	65
Thatcher hard red spring wheat [2]	2.5	79	101	0.4	13	88	69
Second clear (hard red spring wheat) [2]	2.7	71	105	0.6	20	82	78
Granular wheat	3.1	81	98	1.1	9	87	62
White rye	1.5	80	101	0.4	[3]		
Trebi barley	1.0	83	99	0.5	8	72	67
Fulton oats	2.0	74	98	0.6	19	57	81
Illinois Hybrid No. 972 corn	2.5	85	100	1.5	9	54	61
Pink kafir (sorghum)	4.1	92	98	3.4	4	59	30
Colusa rice	1.8	93	97	1.0	4	69	57

[1] The nitrogen content of the starch is expressed, for uniformity, as protein, calculated as $N \times 6.25$ for corn and sorghum and $N \times 5.7$ for the other starches, although the nitrogenous material associated with the starch granules is quite different from the bulk of the flour protein. About 0.02 to 0.04% N (0.1 to 0.3% protein as calculated above) is apparently either incorporated in the starch granules or firmly adsorbed by them, since this is the lower limit of nitrogen in samples of cereal starches prepared by various methods.

[2] Starch centrifuged from suspension of 1 part flour in 6 parts 0.06N NaOH solution. For all other flours 0.03N NaOH solution was used in the same proportions.

[3] Did not precipitate at the flour concentration used.

By-product protein was recovered from the alkaline liquor from the first centrifugation by acidifying to pH 5.5 with dilute sulfuric acid. The precipitated protein was collected by centrifuging and dried. No attempt was made to recover the protein from the starch wash water, which in continuous processing would be used for treatment of the next batch of flour. This wash water usually contained about 10–15% of the total flour protein and would probably augment the recovery of precipitated protein by at least half this amount on recycling.

The results of the application of this process to wheat flour of different grades and from representative varieties of wheat are shown in Table III. The protein content of the crude starch is included as an indication of the quality of starch which would be obtained without any washing.

After one washing, the starch from the straight flours had a protein content of 0.3 to 0.6%, with the exception of that from the No. 2 Dark Northern Spring and the Thatcher hard red spring wheat flours, which contained 1.2% and 0.9% protein, respectively. From these flours, starch with only 0.4% protein was easily prepared by using either an additional 600 parts water (1,200 parts of 0.015*N* NaOH solution) or double the strength of sodium hydroxide (600 parts of 0.06*N* NaOH solution) for the initial flour dispersion. The higher protein content of the starch from the second clear and the granular wheat flour may be attributed to the presence of larger particles of bran or fiber from which the nitrogenous material is not readily extracted.

Starch obtained by this process contains all the alkali-insoluble solids of the flour which lower the starch content to 94 to 98% as compared with a purity of about 99% for the highest-quality starch prepared by the usual commercial methods. The main nonprotein impurities consist of the cellulosic cell-wall fragments and bran. This material, along with the gelatinized starch arising from granules damaged by milling, contributes gumminess or stickiness to the centrifuge cake and prevents ready filtration of the starch. The starch can be separated by centrifugation into prime-quality and lower-quality starch, as is done in commercial rice starch processing (Eynon and Lane, 1928), by taking advantage of the difference in rate of settling of the two fractions.

Tabling Process and its Application to Various Wheat Flours. In the tabling process the starch is recovered in two fractions: (a) the prime-quality starch which deposits on the table, and (b) the "tailings starch" obtained by centrifugation of that part of the suspension passing over the end of the table. An outline of the procedure used for the separation of starch by tabling the alkaline flour suspension is given in the process flow sheet (Fig. 5).

For the preparation of starch, 454 g (100 parts) of flour (moisture-free basis) was added to 5.7 l of 0.03*N* NaOH (1,250 parts aqueous solution containing 1.5 parts NaOH) at 25° to 35°C with rapid stirring. The suspension (about 4.1° Baumé) was run onto a starch table at a rate of about 170 ml per min, the table being 3 inches × 12 ft in size with a pitch of one fourth inch in 10 ft. The tailings were run directly into an imperforate basket centrifuge. One l of water was then run

onto the table at a rate of about 330 ml per min to displace the supernatant layer of alkaline liquor and to sweep away loose solids overlying the starch cake on the table. The starch was removed from the table and the starch milk was adjusted to pH 5.5–6.0 with dilute H_2SO_4 and screened through No. 17 standard bolting silk. The starch milk, made up to a volume of about 3 l (approximately 6.2° Baumé), was

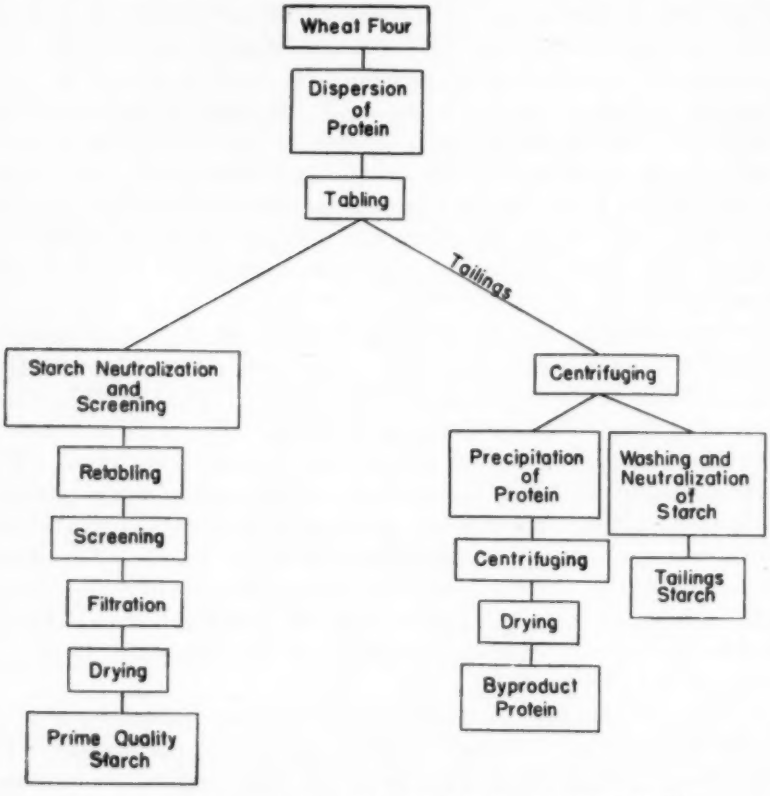

Fig. 5. Tabling process flow sheet for obtaining the products in Table IV.

tabled at a rate of about 330 ml per min. The tabled starch was resuspended with about 800 ml of water, screened through silk as before, filtered by suction on a Büchner funnel, and dried.

The solids from the centrifugation of the first tabling tailings were washed twice by suspension in about 1 l of water and centrifuging, the second washing being accompanied by adjustment to about pH 6.0 with sulfuric acid. Precipitable protein was recovered from the alkaline liquor from the initial centrifuging of the first tailings in the same manner as in the centrifuging process.

The application of the tabling process to flour from different varieties of wheat gave the products shown in Table IV. For brevity, the starch obtained from the second tabling tailings is not included in the tabulation, but usually amounted to about 2–6% of the total starch. The prime-quality table starch constituted between 68 and 83% of the starch originally present in the flour, most of the recoveries

TABLE IV

YIELD OF STARCH AND PROTEIN FROM VARIOUS FLOURS—TABLING PROCESS

(All data calculated on moisture-free basis)

Kind of flour	Tabled starch			First tailings starch			Precipitated protein		
	Yield	Starch recovery	Protein content	Yield	Starch recovery	Protein content	Yield	Protein content	Protein recovery
	Lb/100 lb flour	%	%	*Lb/100 lb flour*	%	%	*Lb/100 lb flour*	%	%
Laboratory runs									
American Banner soft white wheat	67	83	0.2	14	16	0.5	8	77	57
Rex soft white wheat	59	73	0.2	18	19	0.5	4	70	39
No. 2 Hard Winter wheat	57	72	0.3	16	19	0.4	9	84	57
No. 2 Dark Northern Spring wheat	56	71	0.3	19	22	0.4	8	79	46
Thatcher hard red spring wheat	55	72	0.3	18	22	0.5	11	90	62
Second clear (hard red spring wheat)	44	68	0.2	21	26	0.7	16	84	65
White rye [1]	55	72	0.1	20	24	0.6	8	48	42
Trebi barley [1]	50	63	0.2	26	31	0.5	9	68	69
Illinois Hybrid No. 972 corn	67	85	0.5	6	6	2.5	8	56	61
Pink kafir (sorghum)	69	80	0.9	7	7	8.9	5	57	41
Pilot-plant runs									
No. 2 Hard Winter wheat	59	74	0.2	17	20	0.5	9	79	56
White rye [1]	55	73	0.1	22	24	1.2 [2]	—	—	—
Trebi barley [1]	47	60	0.2	31	33	0.7	—	—	—

[1] Starch tabled from a suspension of 1 part flour in 25 parts 0.015*N* NaOH solution. All other tablings were from a suspension of 1 part flour in 12.5 parts 0.03*N* NaOH.
[2] Not washed.

being about 72%. The protein content of this starch was 0.2 to 0.3%, which is comparable to that of the highest quality starch prepared by the usual commercial methods. The first tailings starch fraction, which accounts for 16 to 26% of the starch in the flour, contained 0.4 to 0.7% protein and had a starch content of 88 to 94% when obtained from the straight flours. The first tailings starch fraction from the second clear flour contained only 78% starch as a result of the higher bran and fiber content of this flour, most of this material being recovered in the tailings. The protein recoveries by precipitation were from 46 to 65%, being lower than in corresponding runs by the centrifuging process because of the lower concentration of protein in

the tabling process. The purity of the recovered protein was between 70 and 90%.

The yields of products in the different fractions are representative and were usually reproducible under fixed conditions, within about 2%. The quantities obtained from a given flour are dependent on the rate of tabling, the concentration of flour and sodium hydroxide, the pitch and relative size of the starch table, and other details of manipulation.

Recycling of process liquors is advantageous in general practice. The alkaline liquor from the tailings of the first tabling can be used, with the addition of alkali, for the treatment of at least one further batch of flour before precipitating the protein. If a still higher concentration of alkali solubles is built up by several such recyclings the protein fails to precipitate when the alkaline solution is acidified. The entire tailings from the retabling of the starch can be used for the primary treatment of another batch of flour, care being taken while adding the necessary alkali that the starch in the tailings is not gelatinized by local regions of high alkalinity.

Composition and Properties of Tailings Starch. A typical partial chemical analysis of a washed and neutralized wheat tailings starch, expressed on the moisture-free basis, is given below:

	Percent
Starch	= 93.8 (polarimetric)
Protein	= 0.44 ($N \times 5.7$)
Pentosans	= 1.04
Fatty acids	= 0.65
Ash	= 0.60
Undetermined	= 3.5

Pentosans were determined by the official A.O.A.C. (1940) procedure, the distillate being redistilled and the furfural precipitated by thiobarbituric acid. Fatty acids were determined by acid hydrolysis and extraction from the hydrolysate. The ash content was determined by incinerating the starch at 700°C for 3 hr.

The material not accounted for in the above analysis probably is largely cellulosic in nature.

This tailings starch from the first tabling contains the lighter and often more voluminous alkali-insoluble solids of the flour which fail to deposit on the table. The protein content is very low, in contrast to the tailings or "amylodextrin" fraction obtained in starch production by the dough-washing procedure (MacMasters and Hilbert, 1944a) or the wet milling of wheat (Slotter and Langford, 1944). Microscopic examination of this material showed that it is made up of very small starch granules, gelatinized and swollen starch granules, and cell-wall fragments. This combination of solids offers technical

difficulties in processing. Filtration is slow, if not impossible. Centrifugation in an imperforate basket gives a very soft and gummy cake. When the product is dried in a mechanical convection oven at 40°C, a hard, horny mass is obtained, although vacuum drying at room temperature gives a fairly friable product. Therefore, it is desirable to use the tailings starch while still wet, *e.g.*, for hydrolytic conversion or fermentation, and with as little additional processing as possible for purification.

Pilot-Plant Preparation of Wheat Starch by Tabling. The tabling process used for the pilot-plant preparation of wheat starch was the same as that used in the laboratory. Fifty lb of flour (moisture-free basis) was mixed with 75 gal of water containing 0.75 lb of NaOH. The flour suspension was run onto a starch table, 1 ft × 40 ft in size with a pitch of about 0.5 inch in 10 ft, at a rate of about 0.6 gal per min (0.9 gal/sq ft/hr). The results given in Table IV are in agreement with those for the laboratory processing of the same flour. No difficulties were experienced in this larger-scale application of the procedure.

Applicability to Flour from other Cereal Grains. The centrifuging and tabling processes for recovering starch after alkali treatment were applied to rye, barley, oat, rice, corn, and sorghum flours without modification except in the amount of water used in the initial dispersion of rye and barley flours for tabling. Since these studies were designed to show the effectiveness of the wheat flour processing conditions for other flours, no attempt was made to establish optimum conditions for the individual flours. The yields of products obtained from these flours are shown in Tables III and IV.

The centrifuging process used with rye, barley, and oat flours gave starch comparable in purity to that from wheat flour, the protein content being 0.4 to 0.6%. The starch from corn, sorghum, and rice flours had a higher protein content, 1.0 to 3.4%, due to insolubility of a fraction of the protein in the alkali and/or mechanical retention of protein in vitreous particles of flour which are not broken up during the short treatment with alkali. The protein recovered by precipitation was between 57 and 81% of the flour protein for all but sorghum and rye flours. The low recovery of protein from sorghum flour was due to incomplete dispersion of the protein, since 51% of the flour protein was recovered in the starch cake. The alkaline liquor from the rye flour did not give a precipitate of protein on acidification when the prescribed flour concentration was used. The use of a more dilute dispersion mixture, containing 1 part rye flour in 12 parts 0.03*N* NaOH solution, permitted fairly satisfactory precipitation and recovery of about 62% of the protein in the rye flour. Oat and corn flours ap-

peared to have a higher alkali-binding capacity, since the addition of more alkali was necessary to bring the pH of the mixture above 10.6.

The tabling process, as described for wheat flour, was less applicable than the centrifuging process to other cereal flours. Rice and oat flours could not be processed by tabling because the starch, being composed of very small granules, settled too slowly to permit retention on the table at the rates of flow used. The alkaline protein dispersion from rye and barley flours was rather slimy and viscous, so that a more dilute flour suspension (containing twice the quantity of water used for wheat flour) was necessary to provide satisfactory initial deposition of starch on the table. The retabling of the starch, however, proceeded as described for wheat starch to yield prime-quality starch having the very low protein contents of 0.1 and 0.2%, and representing starch recoveries of 72 and 63%, from rye flour and barley flour, respectively. Corn and sorghum flours gave higher recoveries of starch on the table, but the protein contents were 0.5 and 0.9%, respectively.

The pilot-plant processing of barley and rye flours, using 50 lb of flour in each case and twice the quantity of water used for wheat flour, proved entirely comparable to the laboratory processing, as shown by the results presented in Table IV. Protein recovery was not studied in these runs.

Evaluation of the Alkali Process for Starch Production

The applicability of the alkali process to a wide variety of raw materials permits a choice on the basis of availability and economy. The general success in the preparation of starch from all types of wheat flour studied indicates the feasibility of using such materials as low-grade wheat flour, flour of limited value for baking, such as that from some of the soft white wheats of the Pacific Northwest, and probably flour from damaged grain which is of such quality that it is unsuitable for food. That the starch from damaged wheat is generally of normal quality has been shown by MacMasters and Hilbert (1944). While wheat flour appears to be the most suitable raw material, rye, barley, and oat flours can also be processed with little or no modification of the steps. Less satisfactory results were obtained with corn, sorghum, and rice flours, the separation of protein from the starch not being as complete under the conditions used.

Starch which is well suited for use as such, for conversion to glucose or malt sirups, or for fermentation can be prepared by the alkali process. The quality of the starch is governed by the separation method used and the extent of purification. The tabling process for starch separation and fractionation is adapted to the production of a major fraction of very high-quality starch free of fiber and cell-wall

fragments and having a protein content comparable to that of the best commercial starches. This prime-starch fraction, which is approximately 70 to 80% of the starch in the flour, can be filtered and dried for use as such or converted into other products. The tailings starch is of lower quality, containing fiber, cell-wall fragments, and gelatinized and small starch granules, but relatively little protein. It would preferably be converted to lower quality sirups or used for other purposes requiring wet starch of fair purity.

The centrifuging process is advantageous for the preparation of starch that is to be used in the wet state for purposes not requiring the highest quality starch. By this process, all of the starch in the flour is recovered in a single fraction, the once-washed product usually having a protein content of between about 0.3 and 0.6%.

Protein obtained from wheat is of particular value at the present time for the production of monosodium glutamate which is much in demand as a condiment. Tests by a commercial producer of glutamic acid have shown that the by-product wheat protein from the alkali process is suitable for this purpose. Because of the chemical alteration which has occurred, the recovered protein cannot be used for the fortification of wheat flour in the manner that "native" or "gum" gluten is used.

The alkali process applied to flour provides a relatively simple method for producing starch by the use of conventional types of processing equipment. The recovery of precipitated protein and tailings starch appears to offer technical difficulties which would require study from a chemical engineering viewpoint for the large-scale application of the alkali process. The process, in certain cases, could be employed advantageously in conjunction with existing plant facilities. The production of sirups or sugars by the conversion of starch prepared from wheat or other cereal grain flour in beet-sugar factories or sugar refineries might merit consideration in regions where the raw material supply and cost are favorable. These establishments already have most of the equipment necessary for working up the starch conversion liquors. The utilization of beet-sugar factories in this way also would permit year-around operation of the plant by the production of starch conversion products during the idle period between sugar campaigns.

Summary

As a basis for the production of starch from flour, conditions have been established for the essentially complete dispersion of the protein of wheat flour, without materially affecting the starch, by treatment with dilute aqueous sodium hydroxide. Precipitation of the protein on acidification of the alkaline solution has been studied.

Results of preparation of starch and by-product protein from wheat flour by alkali treatment have been presented. Two representative procedures, centrifuging and tabling, were used for starch separation. The alkali process, as used for wheat flour, has been applied also to barley, rye, oat, corn, sorghum, and rice flours. These raw materials, under the conditions used, were not as satisfactory as wheat flour.

The tabling process yields a prime-quality starch fraction, equivalent to 70 to 80% of the starch in the wheat flour. The remainder of the starch is recovered as a lower-quality fraction which is low in protein (0.4–0.7%) and suitable for certain conversion or fermentation uses.

The centrifuging process permits isolation of all the starch of the wheat flour in a single fraction having a protein content of 0.4–0.6%. Included in this product are the other alkali-insoluble solids which reduce the purity of the starch to between 94 and 98%.

The recovered by-product wheat protein has a purity of 70 to 90% and constitutes 50 to 80% of the flour protein.

The alkali process offers a means of utilizing any variety of wheat, as well as some other cereal grains, as alternatives to corn for maintaining or increasing the production of starch under special conditions of raw material supply and cost.

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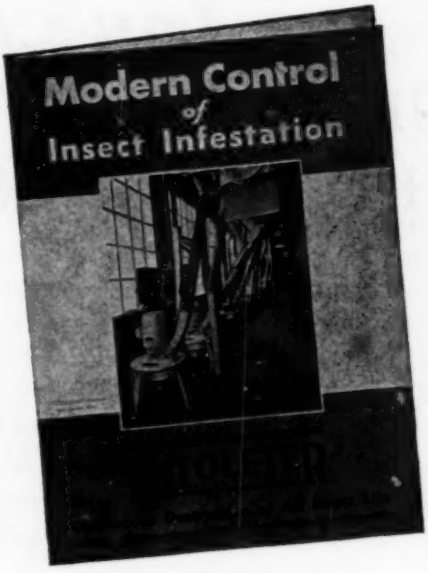

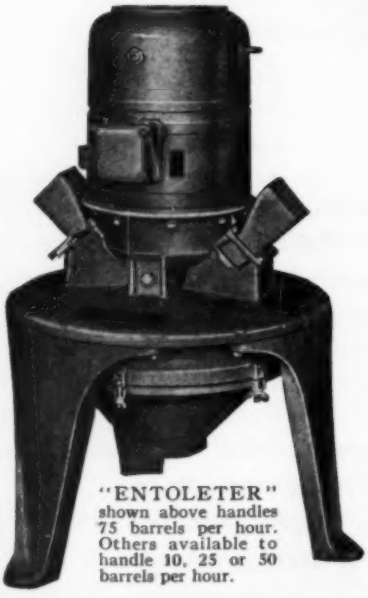

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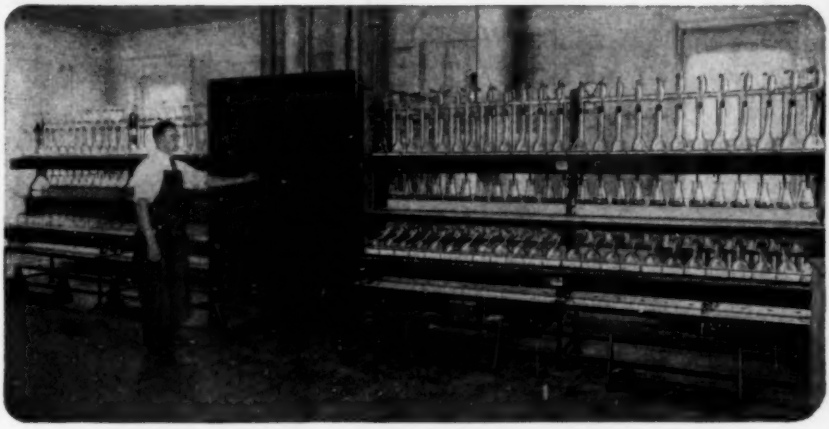

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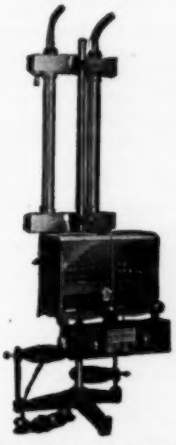

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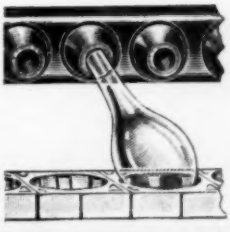

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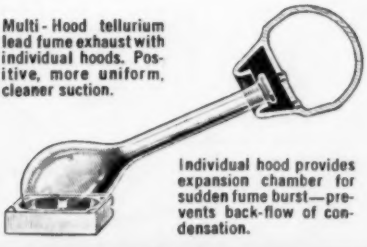

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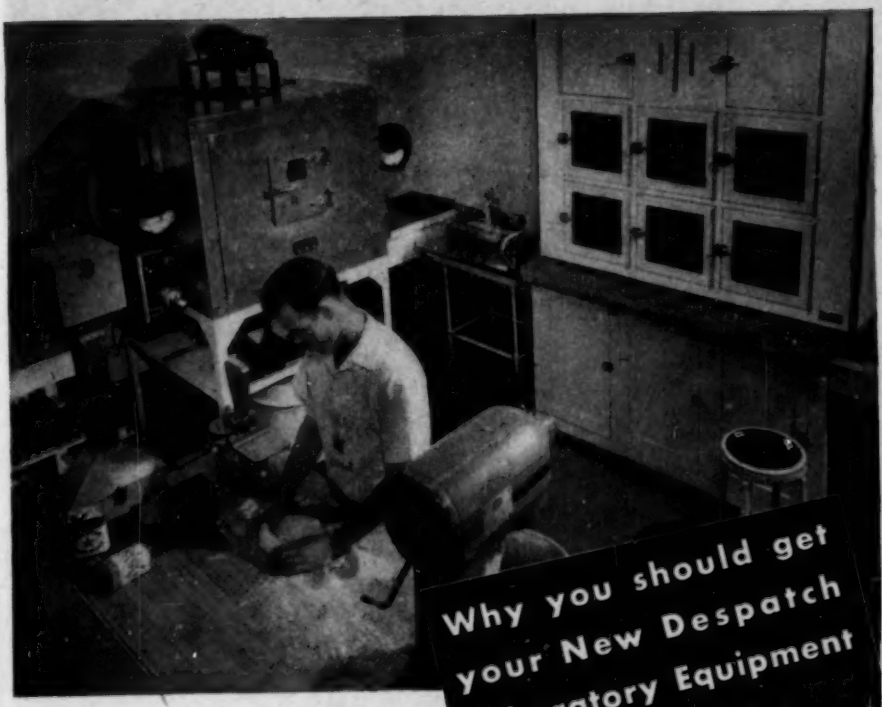

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